17822

TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

- CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section (☐ *Contains CBI*).
Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

1.0 SUBMISSION TYPE - *Contains CBI*

☐ 8(d) **XX 8(e)** ☐ FYI ☐ 4 ☐ OTHER: Specify 8EHQ-0299-14385

X Intial Submission ☐ Follow-up Submission ☐ Final Report Submission

Previous EPA Submission Number or Title if update or follow-up: Docket Number, if any: #

☐ continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e): optional for §4, 8(d) & FYI)	**2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID**	**2.3 FOR EPA USE ONLY**
X- YES ☐ NO	P917-006-894 99-2-16	

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY - *Contains CBI*

Reported Chemical Name (specify nomenclature if other than CAS name):

CAS# : 181274-15-7

2-((((4-Methyl-3-propoxy-4,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl)carbonyl)-amino)-Sulfonyl)-benzoic acid-methyl-ester Na

Purity _97.6__%

X- Single Ingredient

☐ Commerical/Tech Grade

☐ Mixture *Trade Name:* MKH 6561 *Common Name:* Sulfonamide

CAS Number	*NAME*	*% WEIGHT*
Other chemical(s) present in tested mixture		

☐ continuation sheet attached

4.0 REPORT/STUDY TITLE ☐ *Contains CBI*

One-Generation Study in Wistar Rats, Report # 26743

8EHQ-99-14385

continuation sheet attached

99 FEB 23 AM 7:39 RECEIVED OPPT NCIC

5.1 STUDY/TSCATS INDEXING TERMS

[CHECK ONE]

HEALTH EFFECTS (HE): X ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY TYPE:	SUBJECT ORGANISM (HE, EE only):	ROUTE OF EXPOSURE (HE only):	VEHICLE OF EXPOSURE (HE only)
_____	RATS	_____	_____
Other: One-Generation	*Other:*	*Other:* Lung	*Other:*

6.0 REPORT/STUDY INFORMATION ☐ *Contains CBI* X - Study is GLP

Laboratory Bayer AG Toxicology Lab, Wuppertal, Germany Report/Study Date 10/16/97

Source of Data/Study Sponsor (if different than submitter) _____ Number of pages 129

☐ continuation sheet attached

99 MAR 31 AM 9:39 RECEIVED OPPT NCIC

7.0 SUBMITTER INFORMATION ☐ *Contains CBI*

Submitter: Donald W. Lamb Title: VP, Product Safety & Regulatory Affairs Phone: 412-777-7431

Company Name: Bayer Corporation

Company Address: 100 Bayer Road, Pittsburgh, PA. 15205

Submitter Address (if different): _____

Technical Contact: Same as above Phone: ( ) _____

☐ continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS - *Contains CBI*

Note: This substance is an experimental herbicide

☐ continuation sheet attached

Submitter Signature: Donald W Lamb **Date:** 2/16/99

CONTAINS NO CBI

88990000104

9.0 CONTINUATION SHEET

TSCA CBI STATUS:

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Submitter Tracking Number/Internal ID

P917 006 894
99-2-16

Continuation of 2.1

As litter size at birth and the viability index were reduced in the 20,000 ppm dose group, and there was a shift towards a reduced number of males born in the 20,000 ppm dose group, this study is being reported.

Abstract

MKH 6551 was examined for possible effects on reproduction using Wistar rats in a one-generation study, which was performed as a pilot study for a two-generation study. MKH 6561 was administered to groups of 10 males and 10 females at doses of 0 (control), 1000, 5000, or 20,000 ppm in the diet.

There were no compound-related clinical findings and no unscheduled deaths.

There was no compound-related effect on body weight or food consumption.

There were no compound-related gross pathological findings or organ weight effects.

Litter size at birth and the viability index were reduced in the 20,000 ppm dose group. There was a shift towards a reduced number of males born per litter in the 20,000 ppm dose group.

None of the remaining reproduction parameters were affected.

There were no compound-related clinical or gross pathological findings observed in the pups.

In conclusion, the parental NOEL was 20,000 ppm and the reproductive NOEL was 5000 ppm..

STUDY TITLE

MKH 6561
One-Generation Study in Wistar Rats

DATA REQUIREMENT

US EPA-FIFRA Guideline No.: None

AUTHOR

Dr. R. Eiben

STUDY COMPLETION DATE

October 16, 1997

PERFORMING LABORATORY

BAYER AG
DEPARTMENT OF TOXICOLOGY
Friedrich-Ebert-Strasse 217-233
D-42096 Wuppertal
Germany

LABORATORY PROJECT ID

Bayer AG Report No. 26743
Bayer AG Study No. T3060953

STATEMENT OF DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C):

BAYER CORPORATION

Dr. J.H. Thyssen: BP Stuart/for JH Thyssen
Vice President, Toxicology

Date: 2-1-99

GLP COMPLIANCE STATEMENT

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP)* and with the Principles of Good Laboratory Practice according to Annex 1 ChemG ** and meets the FIFRA Good Laboratory Practice Standards (= 40 CFR Part 160), with the exception that recognized differences exist between GLP principles/standards of OECD and FIFRA (for instance authority granted by Agency inspectors and certain record retention requirements).

STUDY DIRECTOR
BAYER AG

Eiben
Dr. R. Eiben

May 7. 1997
Date

SPONSOR
BAYER AG.

Dr. L. Machemer

Oct. 15, 1997
Date

SUBMITTER
BAYER CORPORATION

BP Stuart for JH Thyssen
Dr. J.H. Thyssen
Vice President, Toxicology

2-1-99
Date

* Bundesanzeiger No. 42a (March 2, 1983)(German version)
** Bundesgesetzblatt, Part I (July 29, 1994)

FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

STUDY DIRECTOR
BAYER AG

Eiben
Dr. R. Eiben

May 7, 1997
Date

SPONSOR
BAYER AG

L. Machemer
Dr. L.Machemer

Oct. 15, 1997
Date

SUBMITTER
BAYER CORPORATION

BP Stuart for JH Thyssen
Dr. J.H. Thyssen
Vice President, Toxicology

2-1-99
Date

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TABLES:

Contents of Report Part 1

QUALITY ASSURANCE STATEMENT

Test Substance : MKH 6561

Study No. : T3060953

The study was audited by Quality Assurance on the dates stated below. Audit reports have been submitted in writing to the Study Director and, if necessary, to the laboratory management or other persons affected.

Dates of audit	**Dates of report to study director / management**
Jul. 04, 1996 (study plan)	Jul. 04, 1996
Jul. 12, 1996	Jul. 12, 1996
Aug. 09, 1996	Aug. 09, 1996
Aug. 29, 1996	Aug. 29, 1996
Sep. 19, 1996	Sep. 19, 1996
Oct. 02, 1996	Oct. 02, 1996
Oct. 09, 1996	Oct. 09, 1996

To the best of my knowledge, the study results and methods used have been correctly reported.

Quality Assurance Unit PH-QA-C/GLP, BAYER AG

Date: Sept. 24, 1997

Responsible: .....
(Dr. Niemers)

SIGNATURES

Study Director: Eiben October 16, 1997

(Dr. R. Eiben) (Date)

Head of Carcinogenicity and Genotoxicity: Bomhard Oct. 16, 1997

(Dr. E.M. Bomhard) (Date)

1. SUMMARY

MKH 6561 was examined for possible effects on reproduction of Wistar rats in a one-generation study, which was performed as a pilot study for a two-generation study. This involved administration of **MKH 6561** to groups of 10 male and 10 female rats at concentrations of 0 (control), 1000, 5000, or 20000 ppm in their diet.
F0 animals were pretreated over four weeks and allowed to mate over a period of up to three weeks. F1 offspring were nursed up to an age of three weeks.

Up to 20000 ppm no clinical findings due to the treatment were seen. None of the F0 rats diet unscheduled.

Body weights and food intake of the parent animals were not affected up to 20000 ppm.

No macroscopical findings were made at necropsy of the F0 rats up to 20000 ppm.
There were no changes in organ weights in treated rats.

Litter weight, litter size at birth, number of pups born as well as viability index were reduced at 20000 ppm. There was a shift towards a reduced number of males born per litter at 20000 ppm.
None of the remaining reproduction parameters were affected at concentrations of up to 20000 ppm.
No test substance-related clinical or gross pathological findings were seen in the pups.

Thus, dietary concentrations of up to 5000 ppm **MKH 6561** were tolerated without adverse effects on the offspring under the described conditions.

2. INTRODUCTION

A one-generation study as a pilot study for a two-generation study on Wistar rats with one litter per generation was conducted with **MKH 6561**, a test substance possessing herbicidal properties.

As far as meaningful for a pilot study the following Guidelines were taken into account as far as possible: U.S. EPA (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Series 83-4: "Reproductive and Fertility Effects"; Revised Edition, November 1984) and the OECD (Guidelines for Testing of Chemicals, Section 4: Health Effects, No. 415: "One-Generation Reproduction Toxicity Study", adopted May 26, 1983).
This study includes also the recommendations given in OECD Guideline No. 421 (Reproduction/Developmental Toxicity Screening Test).
Furthermore, this study was conducted according to the „Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration", Society of Agricultural Chemicals, Japan 1985 (MAFF Requirements).

3. GENERAL INFORMATION

Table 1 - Key Study Dates

Study Identification:	
Test Number:	T3060953
Pathology Number:	4802
Receipt of F0 Animals:	July 3, 1996
Animal Age at Delivery:	about 9 weeks
Start of Study (First Day of Treatment):	July 5, 1996
Animal Age at Study Start:	10 - 11 weeks
Initial Weights:	
Males:	265 - 347
Females:	158 - 207
End of Study (Last Animal Necropsied):	October 9, 1996
Total Duration of Study:	14 weeks

3.1 Test Facilities

The experimental phase and the evaluation of the study were conducted at the Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-42096 Wuppertal, Germany.

3.2 Time Schedule in the Tables

The times (days or weeks) stated in the data listings are defined as follows:

a. Body weights, clinical findings and food intake:

Week 0 refers to results obtained before administration commenced on the first day of use. It may also be used to include occurrences during the first week of treatment. Week 1 refers to data recorded on day 8, etc., allowing for a margin of ± 3 days. Days of body weight or food consumption measured during pregnancy and lactation are given in respect to the first day of pregnancy or lactation.

The food intake week/day is designated according to the week/day on which the unconsumed food was weighed. In calculations of mean food intakes the actual number of days over which consumption took place was taken into account.

b. Lists of surviving F0 animals:

These lists indicate the number of animals still alive on the last day of a given week, or the day on which the animal died.

3.3 Archiving

The study protocol, original copy of the Report, study documentation and raw data are held on file by BAYER AG. Materials (test compound, fixed tissue) have also been retained.

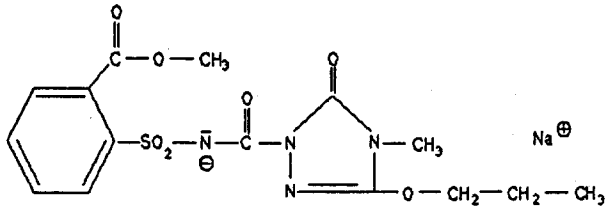
3.4 Persons Involved, Responsibilities

Study Director:	Dr. Eiben
Head of Carcinogenicity and Genotoxicity:	Dr. Bomhard
Analyses of test substance:	Dr. Gau
Analyses of test substance in the diet:	Dr. Rüngeler
Procurement of laboratory animals:	Dr. Hoffmann
Animal housing:	Dr. Eiben
Gross pathology:	Dr. Rinke, Dr. Sander
Technical services:	Mr. Lömker, grad. engineer
Computer-assisted data recording and processing:	Dr. Klotz
Archiving:	Prof. Dr. Schlüter
Quality Assurance:	Dr. Lehn

4. MATERIALS, TEST SYSTEM, METHODS

4.1 Test Substance and Administration

Table 2 - Test Substance and Administration Data

Test Substance:	**MKH 6561**
Producer:	BAYER AG
Chemical Name:	2-((((4-Methyl-3-propoxy-4,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl)carbonyl)-amino)-sulfonyl)-benzoic acid-methyl-ester Na
Structural Formula	
CAS No.:	not yet established
Molecular Mass:	420.4 g/mol
Molecular Formula:	$C_{15}H_{17}N_4O_7SNa$
Batch No.:	05649/0004 (reserve sample is stored)
Purity	97.6% (January 12, 1996)
Released for Toxicity Studies	until December 26, 1996
Appearance:	white powder
Storage:	room temperature
Administration:	
Route and Frequency:	in the diet ad libitum once weekly up to necropsy
Excipient:	1% peanut oil (DAB 10)
Diet:	Altromin® 1321 meal
Storage of Formulation(s):	at room temperature
Formulation Stability:	at least 14 days

4.1.1 Dietary Preparation

MKH 6561 was blended (using a mixing granulator manufactured by Loedige, Paderborn) with Altromin® 1321 containing 1% peanut oil to minimize dust formation (including 0 ppm concentration). The amounts of test substance were calculated on the basis of assumed 100% content of **MKH 6561**. The diet preparations were done weekly.

4.1.2 Analyses of Test Substance in Food

Analytical investigations on homogeneity and stability of the test compound in diet preparations were done prior to commencement of the study using samples from test mixtures (data were taken over from study T2055651). The test substance content in the food given to the animals was checked at regular intervals throughout the study (start of study, 1 randomly selected during the study, end of study). This was done by analyzing samples of the food mixes used. Per dose one sample of the food mixes was taken on the day the food was prepared, and another was taken after being kept under animal room conditions for the feeding period. All samples taken directly after diet preparation or at the end of the feeding period(s) were kept deep frozen (at temperatures of approx. -20°C) until examination.
Reserve samples from each mixture were stored at least for 8 weeks at about -20° C.

4.2 Dosages and Study Groups

The dose schedule (nominal concentrations of **MKH 6561**) and the distribution of animals by study group are summarized in Table 3 on the next page.

Table 3

Dosing Schedule and Group Allocation				
Group No.	Dose ppm	Sex	Number of animals	Animal No.
1	0	m	10	F0 1 - 10
2	0	f	10	F0 11 - 20
3	1000	m	10	F0 21 - 30
4	1000	f	10	F0 31 - 40
5	5000	m	10	F0 41 - 50
6	5000	f	10	F0 51 - 60
7	20000	m	10	F0 61 - 70
8	20000	f	10	F0 71 - 80

Randomization

The F0 animals were randomly allocated to the individual study groups before treatment started. The rats were weighed individually beforehand, divided into two weight classes (light and heavy), and were kept further individually. Using a random list based on evenly distributed random numbers and especially generated for this study the animals were chosen individually from both collectives and assigned to the group specified by the random list. The mean body weights at the start of the study can be found in Report Part 2.

The random list used to appoint F0 rats to groups was produced by using a program from the IBM Scientific Subroutine Package at the Institute of Biometrics, BAYER AG.

The random lists for mating and litter reduction (=culling) were prepared on a HP 3000 computer system using a random-number generator.

4.3. Rationale for Dose Selection

Dose selection was based on results of a subchronic feeding study with dietary concentrations of 0, 250, 1000, 4000, and 20000 ppm (Bomann, W. et al Bayer Report 25597, 1996).

In this study no toxic effect was established in the groups up to 4000 ppm. At 20000 ppm a significant increase in water intake and lower plasma levels of glucose and cholesterol were obvious in females. Additionally, in this group rats exhibited irritative changes in the epithelium of the forestomach.

Thus, the dietary concentration of 20000 ppm could be expected to produce slight parental toxicity.

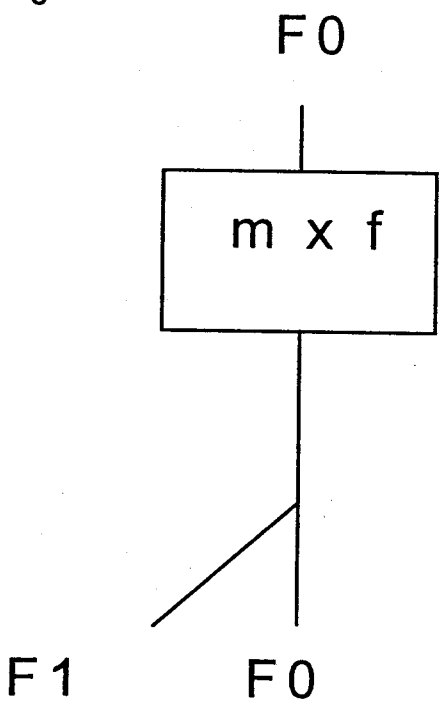
Therefore, it was decided to use the dietary concentrations of 0, 1000, 5000, 20000 ppm in the pilot study.

4.4. Study Organization

The 10-11 weeks old F0 animals used in the study were pretreated with the compound for about four weeks, and then mated. One male and one female of the pertinent group (selected by a random list) were co-housed over night at a maximum of 12 times during the three-week mating period. Inseminated females were not further co-housed. Insemination was established by investigating vaginal smears prepared in the morning. After a gestation period of about 22 days litters were born. If necessary, the F1 litters were reduced (=culled) to eight pups according to random lists four days after birth. The remaining F1 pups were raised to an age of three weeks and then necropsied. F0 animals were killed and necropsied when F1 animals had been weaned.

The following Figure 1 provides a schematic survey of the study organization.

Figure 1



	Duration
Premating period of F0 rats	28 days
Mating period of F0 rats	up to 21 days
Duration of pregnancy of F0 rats	about 22 days
Lactation of F1 pups to an age of three weeks	21 days
Necropsy of F1 pups and F0 females	
Necropsy of F0 males	

4.5. Test System (Animals) and Housing Conditions

4.5.1. Test Animals

This study was conducted with rats, a species recommended in guidelines for reproduction studies. The Wistar rats used were SPF bred of the strain ICO: WU (IOPS Cpb) of the breeder IFFA Credo/France, Belgium. Wistar rats have been used for reprotoxicological studies at Bayer AG for a number of years. Historical data of Wistar rats on the test parameters are available.

The state of health of the breed is monitored and the animals are routinely spot-checked for the main specific pathogens. The results of these tests are filed at Bayer AG. The animals were given no vaccinations or medical treatment.

After receipt the animals scheduled for this study were adapted to the animal room conditions until treatment was initiated, and carefully observed for signs of disease during this time.

Only healthy, symptom-free animals were used for the study. The females were nulliparous and nongravid.

4.5.2 Housing Conditions

During the acclimatization period and the study rats were housed singly under conventional conditions in Type IIa Makrolon® cages, which are larger Type II cages, (as described by SPIEGEL, A., and GÖNNERT, R., Z. Versuchstierkd. 1, 38 (1961) and MEISTER, G., Z. Versuchstierkd. 7, 144 (1965)), on low-dust soft-wood shavings. During the mating period Type III cages were used.

The wood shavings, supplied by Ssniff GmbH, Soest, were tested for contaminants on a random basis (the results are held on file at BAYER AG).
The cages containing the experimental animals were separated by groups and placed on shelves in order of ascending animal number.

The animal room No.: 265 was located in a special building area, separated from the rest by a barrier system. This area could be entered and supplied with materials only through lock systems and a clean corridor. For the disposal of used/soiled material and moribund/dead animals there was a separate transport route. Generally, used or unused cage furniture were moved around in closed containers in this building. To ensure optimal hygienic conditions during the study people entering the barrier system had to change their clothes and to disinfect their hands and shoes.

Identification of the Animals

The parental animals were identified by cage cards stating the study number, test substance, animal number, sex and dose. The color of the cage cards varied according to the dosage group. Additionally, parental animals were identified by ear tattoos. Pups were identified by foot tattoos.

Cleaning, Disinfection and Pest Control

Cages, cage lids, food containers and drinking bottles were cleaned with hot water (no detergents or disinfectants were used) throughout the study. The cage shelves were cleaned and disinfected with Tegol® 2000 at regular intervals.

Cages and food containers were replaced with clean ones weekly. Drinking bottles and caps were changed twice a month.

The floor of the animal room was disinfected once a week (Tegol® 2000). Walls were cleaned regularly in the same way. Continuous pest control was performed using a cockroach trap on pheromone basis. The traps were supplied by Killgerm GmbH, Neuss, placed in the animal room and replaced about monthly by new ones.

Environmental Conditions

The animal room had a standardized climate:

Room temperature:	23 ± 2°C
Air humidity:	55% ± 5%
Light/ dark cycle:	12 hour rhythm from 6 a.m. to 6 p.m. CET (artificial illumination: approx. 140 LUX, for work in the room approx. 380 LUX). From 6 p.m. to 6 a.m. CET orientation light, approx. 3-5 LUX
Air exchange:	approx. 15-20 passages per hour

Occasional deviations from these standards occurred, e.g. during cleaning of the animal room. These did not have any apparent influence on the outcome of the study.

Diet

The diet consisted of a fixed-formula standard diet (Altromin®1321 meal, supplied by Altromin GmbH, Lage) and tap water during the acclimatization period and throughout the study. Food and water were available for the animals ad libitum. The nutritional composition and contaminant content of the standard diet were routinely checked and analyzed on a random basis. The tap water complied with German drinking water standards[1)]. The results of the analyses of the diet and water are held on file. The data available provided no evidence of any effect on the study objective. The food was provided in stainless steel dispensers. Water was supplied in polycarbonate bottles with a capacity of approx. 300 ml or 700 ml (as described by SPIEGEL, A. and GÖNNERT, R., Z. Versuchstierkd. 1, 38 (1961) and MEISTER, G., Z. Versuchstierkd. 7, 144 (1965)).

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1) German Drinking Water Ordinance of Dec. 5,.1990, Federal Legal Gazette No. 66, issued on Dec. 12.,1990, page 2612

4.6. General Investigations

4.6.1 Inspection of Animals

All experimental animals were inspected twice daily (once daily on weekends and public holidays), and any findings observed were recorded. In F0 rats a detailed evaluation of the general condition was made and recorded at the weekly change of cages during the premating period. During gestation period females were examined on day 0, 7, 14, 20, and during the lactation on day 0, 4, 7, 14 and 21. The general state of health, behavior, condition of the fur, and the body openings as well as excretory products were examined in this evaluation. Findings and abnormalities were entered online or offline using a coding system and uncoded/free text. For pup inspections see Chapter 4.7.2.

4.6.2 Determination of Body Weight and Food Intake of Parent Animals (F0)

All F0 animals were weighed at the start of the study. The male animals were weighed at weekly intervals up to week 4 (premating period). Body weights of F0 females were recorded weekly during premating period, during gestation on postcoital days 0, 7, 14 and 20, and on days 0, 4, 7, 14 and 21 after birth of the pups. Male and female F0 animals were weighed on the date of necropsy to permit calculation of the relative organ weights.

The food intake of F0 rats was measured as follows :
In males measurements were done weekly during the premating period.
In females determinations were performed weekly during the premating period. Furthermore, food intake was recorded on postcoital days 7, 14 and 20 as well as on days 4 and 7 after delivery of the litter.

The food intake was recorded by weighing the quantity of food provided and back-weighing the amount which remained unconsumed.

From these primary data the following was calculated:

a) daily food intake per animal
b) mean daily food intake per animal

for the premating period in total -

c) mean food intake per animal and day
d) mean food intake per animal kg body weight and day
e) cumulative food intake per animal
f) cumulative food intake per kg body weight

Furthermore, from these primary data the following was calculated for the total premating period:

g) cumulative test substance intake per animal
h) cumulative test substance intake per kg body weight
i) mean test substance intake per animal and day
j) mean test substance intake per kg body weight and day

The algorithm used for calculating intake of food and test substance is described in Report Part 2.

The body weight and food consumption values, if any, of females found to be sperm-positive but failed delivering of pups were not reported.

4.7. Reproduction Parameters

4.7.1 Determination of Insemination Rate and Duration of Pregnancy

In each case, the rats were mated overnight during the period from 4 PM to 8 AM. To determine the date of insemination, vaginal smears were taken from the females on the morning following each mating until evidence of copulation was observed. The date at which sperms were found by microscopical examination or a vaginal plug was detected was taken as gestation day 0 in calculating the gestation period. Females which exhibited marked weight gains although insemination had not been established were not further co-housed. No gestation length could be determined for these animals.

The vaginal smears were obtained using a flame-sterilized platinum loop, and were then plated out on slides. The smears were stained for about one minute in May-Grünwald solution, and then microscopically examined.

4.7.2 Recorded Data on Pups

The numbers of live and dead pups as well as the sex of the pups (including those of dead pups if possible) were determined shortly after birth (on postpartum day 0), day 4 (before and after culling), 7, 14 and 21. At these time points (not after culling) individual body weights and clinical signs were recorded as well. Note was taken of any apparent malformations.

4.7.3 Calculation of Indices

The following indices were calculated for each dose group: insemination index, fertility index, gestation index, live birth index, viability index and lactation index. The following formulas were used:

$$\text{Insemination index (\%)} = \frac{\text{No. of sperm-positive females*}}{\text{No. of females co-housed with males}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. of pregnant females}}{\text{No. of sperm-positive females*}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\text{No. of females with live pups}}{\text{No. of pregnant females}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\text{No. of live pups at birth}}{\text{total No. of pups born}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{No. of live pups on day 4 pre-culling}}{\text{No. of live pups born}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{No. of live pups after three/four weeks}}{\text{No. of live pups after four days (after culling)**}} \times 100$$

* including pregnant females that were not sperm-positive.

** moribund pups died during the course of culling were not included

4.8 Necropsies

4.8.1 Necropsy of Parent Animals

After the F1 pups had been weaned, the dams were anesthetized with diethylether and killed by exsanguination and examined for gross pathology.
In F0 females implantation sites were counted and documented but not reported here. In some cases this was done after the uterus had been stained with ammoniumsulfide.
F0 males were killed as scheduled under diethylether narcosis when they were not required for further treatment.

The following organs/organ specimen of the F0 animals were fixed in buffered 10% formaldehyde solution: liver, pituitary gland, vagina, uterus, ovaries, mammary gland with skin, coagulation glands, seminal vesicles, prostate, tattooed ears and all organs/organ specimen exhibiting macroscopic changes.
The testes and epididymides were fixed in Davidson solution.

4.8.2 Necropsy of Pups

Unscheduled Necropsies
Unless autolysis or cannibalism rendered examination impossible, pups that were found dead at birth, that died during the course of lactation as well as those killed (with carbon dioxide) in moribund condition were macroscopically inspected after opening the body cavities, with particular attention being paid to the organs of reproduction.

A lung flotation in tap water was performed during the necropsy of pups found dead on the day of the first litter inspection. This was done to determine whether pups had breathed at birth or not.

Scheduled Necropsies

The pups selected for litter reduction were killed with carbon dioxide on postpartum day 4. Weanlings were killed under diethyl ether anesthesia by head dislocation on postpartum day 21. Both, pups selected for culling as well as weanlings were examined for macroscopical alterations.

Fixation

Macroscopically changed organs, if any, were fixed in buffered 10% formaldehyde solution.

4.8.3 Organ Weights of F0 Rats

The following organs of the routinely necropsied parent animals were weighed:
Brain, liver as well as kidneys, testes and ovaries in pairs.

4.9 Statistics and Presentation of Results

Statistics

The following methods were employed to test for statistical significance:

a. The Dunnett-Test in connection with a variance analyses was used for evaluation of the
- Body weights of parent animals
- Organ weights

b. A Kruskal-Wallis-Test with a Steel-Test was done on food consumption data

These calculations were performed using an HP 3000 computer system.

c. The U-Test was used for the evaluation of the
- Pup weights
- Litter sizes
- Litter weights

These calculations were performed using an HP Vectra personal computer (NPAR 1 way from SAS). The mean pup weight of each individual litter was used as a basis for calculation of the pup weight means of the dose groups. The litter size calculation was based on the number of female animals with live pups.

d. Fisher's exact probability test (two-tailed) at significance levels of a = 5 % and a = 1 % was used for the evaluation of the
- Insemination index[1]
- Fertility index[1]
- Gestation index[1]
- Live birth index[1]
- Viability index[1]
- Lactation index[1]

---

[1] *done if dose-related changes had occurred*

These calculations were performed using an HP 3000 computer system and were calculated and statistically evaluated on a litter basis.

Recording of Data

Body weights, food consumption and clinical findings (if to be determined) were recorded online during premating period. During gestation and lactation these data were processed offline. Organ weights of F0 rats were recorded online or offline. Inlife pup data with the exception of clinical findings were recorded offline. All other data (belonging to adults or offspring) were documented without any computer assistance.

Presentation of Data

Individual pup weights listed under „day 4 after culling" are taken from the same measurement as weights listed under „day 4 before culling".

Data not Presented

Body weights and/or feed consumption data of females not found to be sperm positive or failed living pups and those of sperm-positive but not pregnant females are not reported, since they are not useful for study evaluation. Cumulative feed and substance data were calculated for the premating period only, because varying feed intake data recorded during mating procedure, pregnancy and/or lactation cannot be included in the calculation of cumulative mean values. Individual food intake data recorded during pregnancy or lactation are not presented.

Since no adverse effect were seen on fertility in this study data concerning the number of implantation sites are not reported.

5. RESULTS

The results of the investigations are summarized below. Only group means are given in the tables of Report Part 1. Individual animal data and group means with statistical data can be found in Report Part 2. The individual results of the gross pathological investigations are presented in Part 2 of this Report. For the list of abbreviations used in the tables see Chapter 7.

5.1 Analyses of Test Substance in the Diet

All data of analytical investigations are given in detail in the analytical report to be found in Part 2 of this Report.
Before the start of the study homogeneity and stability of **MKH 6561** in the diet were examined using sample mixtures. The results revealed that the test substance was homogeneously distributed in the diet amount used, and stable in the concentration range used throughout the feeding period (1 week).
Three samples of diet mixtures (start of study, 1 randomly taken during the study, end of study) fed to the animals were analyzed for their content of **MKH 6561** and stability over one week. All food mixtures checked during the study proved to be in the specified concentration range.

5.2 F0 Generation

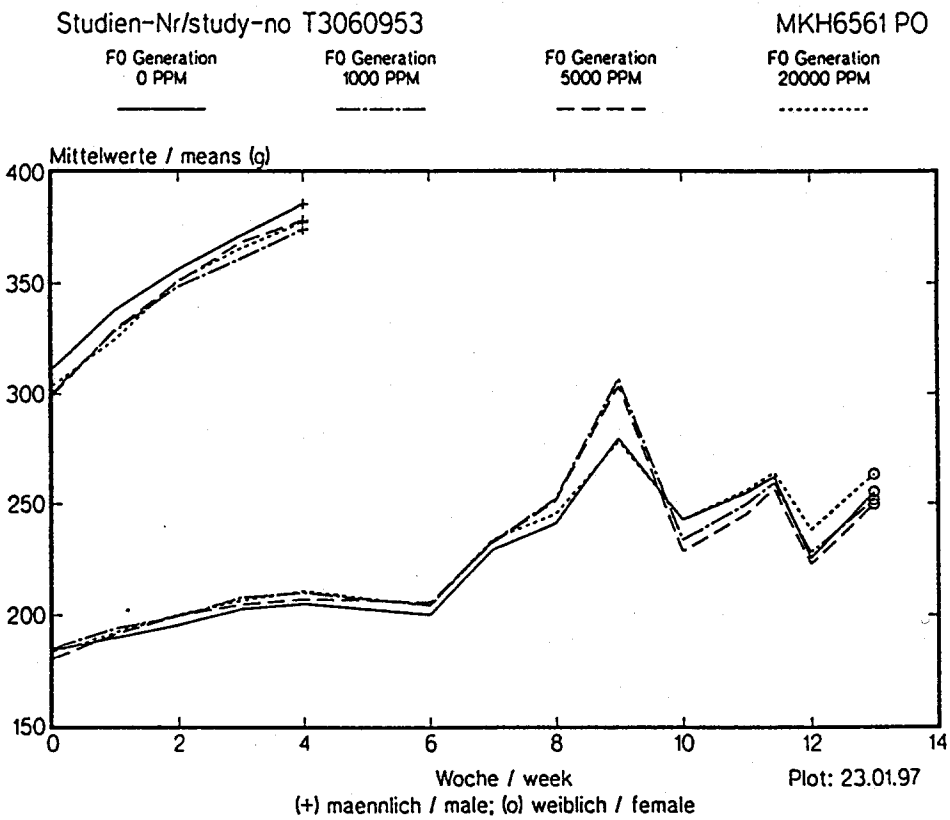
5.2.1 Clinical Signs and Mortality

As can be seen from individual clinical findings presented in Part 2 no test substance-related effects on the appearance or behavior were observed in male or female F0 animals at levels of up to 20000 ppm. There is no evidence of treatment-related mortality in male and female F0 animals at doses of up to and including 20000 ppm, since no animal died or was killed in moribund condition (see survival tables in Part 2).

5.2.2 Body Weights of F0 Parent Animals

The group mean body weights of the male and female F0 rats are plotted against the time of the study in the following Figure 2.
As also shown in the tables with statistics in Report Part 2, the body weights of the treated rats did not differ in a dose-correlated way from those of the control groups during the premating, gestation or lactation period.

Figure 2 Body Weights of F0 Parent Animals



5.2.3 Food Consumption and Test Substance Intake of F0 Parent Animals

The following Table 4 lists the mean food intake per animal and day, per kg body weight and day, and the corresponding cumulative consumption figures for each study group during the premating period. Individual data and the mean consumption figures determined for the individual study weeks are presented in Report Part 2.

During the premating period, the food intake of the F0 rats per animal and day did not differ from the control data to a toxicologically significant extent up to 20000 ppm.

Table 4

Mean Daily and Cumulative Intake of Food during the Premating Period

Dose			g/Animal		g/kg Body Weight	
ppm	Sex	Days	Total	per Day	Total	per Day
0	m	28	588	21.0	1622	57.9
1000	m	28	597	21.3	1694	60.5
5000	m	28	597	21.3	1678	59.9
20000	m	28	610	21.8	1722	61.5
0	f	28	456	16.3	2294	81.9
1000	f	28	394	14.1	1943	69.4
5000	f	28	431	15.4	2151	76.8
20000	f	28	438	15.6	2159	77.1

Table 5 lists the mean test substance intake per animal and day, per kg body weight and day, and the cumulative intake for each study group during the premating period. As can be seen from Table 5, the intake of **MKH 6561** in the treatment groups roughly corresponds to the theoretical dose intervals.

Table 5

Mean Daily and Cumulative Intake of Test Substance during the Premating Period

Dose			mg/Animal		mg/kg Body Weight	
ppm	Sex	Days	Total	per Day	Total	per Day
1000	m	28	597	21.3	1694	60.5
5000	m	28	2985	106.6	8388	299.6
20000	m	28	12206	435.9	34431	1229.7
1000	f	28	394	14.1	1943	69.4
5000	f	28	2157	77.0	10755	384.1
20000	f	28	8753	312.6	43181	1542.2

5.2.4 Gross Pathological Changes in F0 Parent Animals

No gross pathological findings were made at necropsy of male or female F0 animals at levels of up to and including 20000 ppm.

5.2.5 Organ Weights of F0 Parent Animals

The following Table 6 presents the absolute and relative weights of brain, liver, kidneys, testes and ovaries of F0 parent animals as means. Individual animal results are given in Report Part 2.

Table 6

Absolute Organ Weights						
Dose	Sex	Body W.	Brain	Liver	Kidney	Testes
ppm		g	mg	mg	mg	mg
0	m	448	1959	15135	2594	3427
1000	m	442	1994	15219	2464	3371
5000	m	446	1937	15386	2370+	3430
20000	m	444	1938	14242	2502	3530
						Ovaries
0	f	254	1777	11317	1595	112
1000	f	258	1741	13120	1685	104
5000	f	254	1719	12675	1802+	103
20000	f	265	1753	11590	1724	123
Relative Organ Weights						
Dose	Sex	Body W.	Brain	Liver	Kidney	Testes
ppm		g	100 mg/kg Body Weight			
0	m	448	438	3380	579	767
1000	m	442	453	3442	560	764
5000	m	446	436	3450	532+	773
20000	m	444	437	3209	563	798
						Ovaries
0	f	254	701	4412	628	44
1000	f	258	678	5099	654	40
5000	f	254	679	4986	711+	41
20000	f	265	663	4357	650	46

There were no remarkable changes in organ weights measured. Deviations in absolute and relative kidney weights ($p \leq 0.05$) at 5000 ppm are of no toxicological relevance, since they are not dose-correlated and point into different directions in males and females. Therefore, they are considered to be incidental.

5.2.6 Parameters of Reproduction in F0 Parent Animals

5.2.6.1 Insemination Index, Fertility Index, Gestation Index and Duration of Pregnancy

After a premating period of about 4 weeks, pairs of one male and one female F0 animal of the pertinent group were mated. The calculated indices of insemination, fertility and gestation, as well as the mean duration of pregnancy are listed in the following Table 7.

Table 7

Dose (ppm)		0	1000	5000	20000
Insemination index	%	100	100	100	100
Fertility index	%	80	100	100	80
Gestation index	%	100	100	100	100
Gestation length	Days	21.8	22.3	22.5	22.3
Mated females	Number	10	10	10	10

The insemination, fertility and gestation indices as well as the mean duration of pregnancy of treated rats did not differ from the pertinent control data to a toxicologically significant extent.

The mating performance of the F0 animals is given by numerical values in percent listed in the Table 8. Only those mated females were included in which the mating date could be determined by detection of sperms in the vaginal smear.

The mating performance was not affected by the treatment at levels of up to 20000 ppm.

Table 8

F0 Mating Performance				
Day of Mating period	Cumulative Percentage of Sperm-positive Females			
	Dose			
	0 ppm	1000 ppm	5000 ppm	20000 ppm
1	30	30	50	50
2	60	60	80	60
3	80	90	90	
4		100		80
5				
6				
7				90
8				
9				
10				
11	90			
12			100	

5.2.6.2 F1 Litter Parameter at Birth

The following Table 9 lists the total numbers of pups born, those which were found dead at birth, the live birth index, the relation of male and female pups, and the litter size at birth.

Table 9

Dose	Number of Pups		Live Birth	Males	Females	Mean
ppm	Total	Dead	Index %	%	%	Litter Size*
0	94	0	100	52	48	11.75
1000	114	2	98.5	59	41	11.20
5000	119	1	99.2	55	45	11.80
20000	72	0	100	38	62	8.87

* viable pups only

The number of born pups and that of stillbirths, the live birth index, the percentages of male and female pups as well as the litter size at birth were not reduced at levels of up to 5000 ppm.
As an incidental result 5000 ppm dams had born about 20 % more pups compared to those of the 0 ppm group. In the high dose group fewer pups (total and per litter) ($p>0.05$) were born compared to the control group.
There was a slight shift in the male to female relation towards the female sex at 20000 ppm.

5.2.6.3 Clinical Observations in F1 Pups

No significant clinical findings were made in the F1 pups during the three-week lactation period at levels up to 20000 ppm. Malformations were not observed.
The individual pup findings are given in a table in Report Part 2.

5.2.6.4 Body Weights of F1 Pups

The Table 10 lists the mean litter weights at birth and weaning. There was no indication of a treatment-related reduction in litter weights up to 5000 ppm. The marginally lower means at birth and day 21 seen at 5000 ppm are considered as a result of the relatively high number of pups born in this group. At 20000 ppm decreased litter weights were found at birth possibly due to the treatment.
As can be seen from Table 11 there was no dose-dependent reduction in pup weights up to 20000 ppm. The slightly lower pup weights seen in the group 5000 ppm (females $p \leq 0.05$ on day 7) can be explained by the relatively high number of pups born in this group.

Table 10

Dose ppm	Mean Litter Weight in g	
	At Birth	Day 21
0	68.20	320.97
1000	66.42	314.41
5000	65.28	289.40
20000	51.30	288.52

Table 11

Mean Pup Weight During Lactation						
Dose (ppm)	Sex	Day 0	Day 4 after culling	Day 7	Day 14	Day 21
0	m	6.00	9.86	15.61	25.48	40.77
1000	m	6.14	10.11	15.74	25.72	40.74
5000	m	5.73	9.10	14.27	23.37	37.55
20000	m	6.26	10.41	15.76	25.68	40.57
0	f	5.63	9.52	15.20	25.31	40.72
1000	f	5.79	9.71	15.05	25.01	39.82
5000	f	5.39	8.81	13.53+	22.60	36.50
20000	f	5.77	10.04	15.30	25.29	39.57

5.2.6.5 Viability Index and Lactation Index of F1 Pups

The following Table 12 contains data on the lactation performance of the female F0 dams up to postpartum day 4 (viability index) and up to postpartum day 21 (lactation index).

Table 12

Dose ppm	Viability index % Day 4	Lactation index % Day 21
0	99.0	100
1000	100	98.8
5000	92.6	97.5
20000	85.9	100

The viability and lactation indices did not differ to a toxicologically significant extent from the pertinent control figures up to the concentration level of 5000 ppm. In the 20000 ppm group fewer pups had survived at day 4 p.p.

5.2.6.6 Gross Pathological Changes in F1 Pups

Individual findings are listed in Report Part 2.

No treatment-related gross pathological findings were made in F1 pups at necropsy. No skeletal deviations were determined in the F1 pups which died before postpartum day 4, or were killed in the process of culling on postpartum day 4, at levels up to and including 20000 ppm.

6. DISCUSSION AND EVALUATION OF THE RESULTS

MKH 6561 was examined for possible effects on reproduction in a one-generation study in Wistar rats with one litter. This involved administration of **MKH 6561** to groups of 10 male and 10 female rats at doses of 0 (control), 1000, 5000 or 20000 ppm in their diet. This study was used for dose range finding for a subsequent two-generation study.
None of the F0 rats died unscheduled. The appearance and behavior of the F0 parent animals showed no treatment-related effect at levels of up to 20000 ppm.
The body weight development and food intake data of treated F0 animals were comparable with those of controls.
No gross pathological findings were made at necropsy of the parent animals at levels up to and including 20000 ppm.
There were no organ weight differences indicating a compound-related effect.
The reproduction parameters: insemination index, fertilization performance, fertility index, gestation index, gestation length, number of dead pups at birth (live birth index), pup weights and lactation index were not adversely affected at levels of up to 20000 ppm. The total number of pups, the mean litter size and weight as well as the sex relation of pups at birth were not affected up to 5000 ppm. At 20000 ppm litter weights were reduced as result of reduced litter sizes. In addition, in this group there was a shift towards a reduced number of males per litter.
The viability indices were comparable up to 5000 ppm with those of controls, but slightly reduced at 20000 ppm.
No test substance-related clinical or gross pathological findings were observed in the pups. The skeletal development in the pups up to postpartum week 3 was unaffected at levels of up to and including 20000 ppm.

Thus, **MKH 6561** exhibited no adverse effects on reproduction at concentration levels of up to 5000 ppm.
Therefore, for the subsequent two-generation study the dietary concentrations of 0, 100, 4000 and 16000 ppm were chosen.

7. ABBREVIATIONS

aft. ..... after
anim.No./anim.no. ..... animal number
Appl/adm..... application/administration
bef. ..... before
BODY W. ..... body weight
wf; f ..... female
m ..... male
M ..... mean
Max. ..... highest value
Med. ..... median
Min. ..... lowest value
n ..... number of litters examined
N ..... number of animals examined
n.s. ..... not significant
p.c. ..... post coitus
PO..... oral
p.p. ..... postpartum
red..... reduction (=culling)
S.D./s ..... standard deviation
Studien-Nr./study no.. study number
TS 1 % ..... test result at significance level of α = 1 %
TS 5 % ..... test result at significance level of α = 5 %
0 ..... test not applicable
+ ..... difference against control for $p \leq 0.01\%$ significant
++ ..... difference against control for $p \leq 0.05\%$ significant
ns ..... not statistically significant

MKH 6561

One-Generation Study in Rats

Pilot Study for a Two-Generation Study

by

Dr. R. Eiben

T3060953

Part 2 of 2

Contents of Report Part 1

Contents of Report Part 2

Contents of Report Part 2

Bayer AG
PF-PM/NP

09.01.97

Approval of Active Ingredient Sample

Active Ingredient Sample TOX 4015

Sample: *MKH 6561*

Development-No.: *0142835*

Indication: *Herbicide*

Mixed Batch No.: *05649/0004*

Origin of sample: *PF-P/VE*

Responsible Analyst: *Dr. Gau* **Laboratory:** *PB-A*

Analytical Methods: *HPLC, int. Std.*

Approvals:

TOX	Purity		Approved until	Date of Analysis	Comment
4015-00	*97.6*	*%*	*12.07.96*	*12.01.96*	
4015-01	*98.0*	*%*	*26.12.96*	*26.06.96*	
4015-02	*98.0*	*%*	*06.07.97*	*07.01.96*	

(Dr. Haug, PF-PM/NP)

A reserve sample will be retained.

BAYER AG
PH-PD TOXICOLOGY
FRIEDRICH-EBERT-STR. 217-333
D-42096 WUPPERTAL

MKH 6561

Analytical Method Validation
Homogeneity, Stability Data, and Dose Verification
in Animal Ration

ANALYTICAL REPORT

Dr. W. Rüngeler

Study-No.: T3060953

1. TABLE OF CONTENTS

2. SIGNATURES

Head of Analytical Laboratory: _____ April 1, 1997

Dr. W. Rüngeler Date

Occupational Toxicology: _____ Aug. 13, 1997

PD Dr. Dr. J. Pauluhn Date

3. ABSTRACT

A liquid chromatographic method for the quantification of MKH 6561 in animal ration was developed. This work was conducted for tests on stability, homogeneity, and the results of the analyses for verification of nominal concentration of this test compound in mixtures fed to animals. The method, its validation, and the analytical results were presented in this report.

MKH 6561 was extracted from animal ration with acetonitrile/Milli-Q-water (9:1/v:v). It was analyzed on a reversed phase (C18) column and 230 nm ultraviolet (UV) detection.

Recovery of MKH 6561 from rodent ration fortified with 250 and 20000 ppm ranged from 97.2% to 90.7% with a mean recovery value of 93.9%. The limit of quantification for MKH 6561 was approximately 14.4 µg/ml. Under the conditions of this method, the limit of reliable measurement of MKH 6561 was approximately 100 ppm for rodent ration.

The analytical data verify that the test material is homogeneously distributed and chemically stable within the concentration range of 100 ppm to 20000 ppm. Under current sample preparation and handling conditions stability in the diet was assured for a period of at least 14 days.

The test material content in the toxicology test mixtures, prepared during the study, agreed with the target concentrations within defined limits.

4. INTRODUCTION

A liquid chromatographic method for quantifying MKH 6561 in animal ration was developed. This work was conducted for tests on stability, homogeneity, and for verification of nominal concentration of this test compound in these mixtures. The method, its validation, and the analytical results were presented in this report.

MKH 6561 was blended with Altromin 1321, which was mixed with 1% peanut oil (DAB 10). For analytical investigations, representative samples, produced under the study director's responsibility, were taken at different points during the study from the diet fed to animals. These samples were extracted and diluted with acetonitrile/ Milli-Q-water (9:1/v:v) and after filtration subsequently quantified by high-performance liquid chromatography (HPLC) with UV-detection (DAD; wavelength: 230 nm). Standard solutions of approved MKH 6561 were used as a basis for evaluation.

For the analytical quantification of the test compound a concentration range of 14.4 to 208.0 µg/ml was covered. The calibration curve, produced from standard solutions, was prepared anew for each analytical sequence. The linearity of the calibration curve, however, must be given. Essentially, all sample concentrations were always within the calibration range documented for each sample sequence.

The experimental standard of this part of the study conforms to the OECD Principles of Good Laboratory Practice (GLP) and to the Principles of Good Laboratory Practice (GLP) according to Annex 1 ChemG {Bundesanzeiger No. 42a of the 2nd of March 1983 and Bundesgesetzblatt Part I of the 29th of July 1994}.

Investigations necessary for drafting the analytical method and performing analyses were conducted in July 1996 to October 1996 at the Department of Industrial Toxicology, Institute of Toxicology of Bayer AG, D-42096 Wuppertal-Elberfeld, Friedrich-Ebert-Strasse 217-333.

The protocol, raw data, and the final report were archived in locations specified by Bayer AG, in accordance with GLP requirements:

Study-No.: T3060953

5. RESPONSIBLE PERSONS

Head of the Department of Industrial Toxicology: ..... Prof. Dr. E. Löser

Analysis: ..... Dr. W. Rüngeler

Study Director (Toxicology): ..... Dr. R. Eiben

Archiving of the experimental data: ..... Prof. Dr. G. Schlüter

Quality Assurance: ..... Dr. H. Lehn

6. MATERIALS AND METHOD

6.1. TEST SUBSTANCE

Test material:	**MKH 6561**
Mixed Batch No.:	5649-0004
Purity:	98.0%
Origin of sample:	Bayer AG, PF-P/VE
Stability approved until:	Dec. 26, 1996
Test material storage:	room temperature
Stability of analytical samples:	was ensured throughout the test period
Toxicology feed mixtures:	in Altromin 1321; mixed with 1% peanut oil DAB 10

6.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

6.2.1. Instruments

Extraction: PTFE column
High performance liquid chromatograph HP1090 equipped with **
- Autosampler
- DAD (Diode array Detector)
- Integration: HP 3365 DOS-WorkStation/ChemServer **
supplied by Hewlett-Packard Inc.
Standard laboratory equipment and glassware
{Gas tight} Syringes (25 µl; 100 µl; 250 µl; 10 ml ; Hamilton) **

6.2.2. Method

Column:	Hypersil ODS	5 µm; L = 125 mm; ID = 4 mm; Merck/Grom	**
Oven temperature:		off	
Flow rate:		1.00 ml/min	
Mobile phase:	A:	buffer solution	
	B:	acetonitrile	
	gradient program:	time 0.0 --> %B=20 (start conditions)	
		time 2.0 --> %B=20	
		time 10 --> %B=70	
		time 12 --> %B=90	
Injection volume:		20.0 µl (Autosampler)	
Detector:	wavelength:	230 nm	
	band width (BW):	8 nm	
	reference:	450 nm / 80 nm BW	**

6.3. SOLVENTS AND CHEMICALS

Acetonitrile Lichrosolv; Merck No. 30 **
Phosphoric acid (85%); H_3PO_4 ; Merck **
Sodium sulfate; Merck No. 6649 **
Sea sand; Riedel de Haen No. 18649 **
Deionized water (Milli-Q-water), available from Millipore unit, Fa. Millipore
Buffer composition: 1.5 ml H_3PO_4 ad 1000ml Milli-Q-water

** or equivalent

7. SAMPLE PREPARATION

The animal rations were prepared under the study director's responsibility. For sample preparation 10 g of test material were mixed with 20 g of a mixture of sodium sulfate/sea sand (1:1/w:w) and filled in an empty PTFE column. 100 ml of acetonitrile/Milli-Q-water (9:1/v:v) were used for extraction, the extracts were filled in a volumetric flask, diluted and brought up to volume with solvent. These solutions were injected onto the HPLC after appropriate dilution.

8. CALIBRATION OF THE ANALYTICAL METHOD

To set-up the calibration series, test material solutions in acetonitrile were prepared with appropriate concentrations. The stability of these solutions was checked at room temperature over a period of 9 days. No decrease in concentration was observed (raw data presented in study No. T0058151).
The method-specific parameters were adjusted on the HPLC instrument. 20.0 µl of each calibration concentration was injected for preparation of the calibration curve.
Measurement wavelength: 230 nm (UV spectrum see fig. 1)
Fig. 2 showed a typical chromatogram of these external calibration solutions and additionally a food extract chromatogram. A statistically evaluated calibration curve was shown in Fig. 3. This curve was plotted by the integrator and based upon the injected concentrations. The calibration line was plotted anew for each analysis sequence, and deviations from this calibration range were therefore possible. All sample concentrations were always within the calibration range documented for each sample sequence. The quantitative evaluation was performed by determination and comparing the peak area of **MKH 6561** of the analytical solution with the peak areas of the external standard solutions.

Retention time: **MKH 6561** approx. 8.5 min; concentration range: 14.4 to 208.0 µg/ml

14.4 µg/ml was the limit of quantification of the analytical measurement using this method.

Figure 1: UV-spectrum of MKH 6561 (raw data presented in study No. T0051010)

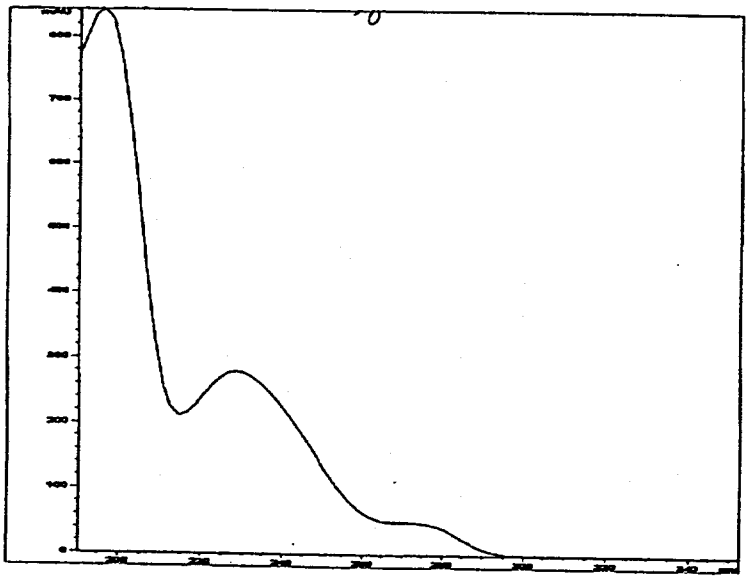


Figure 2.1: typical LC-chromatogram of the test substance as a calibration standard
test material concentration: 52.0 µg/ml

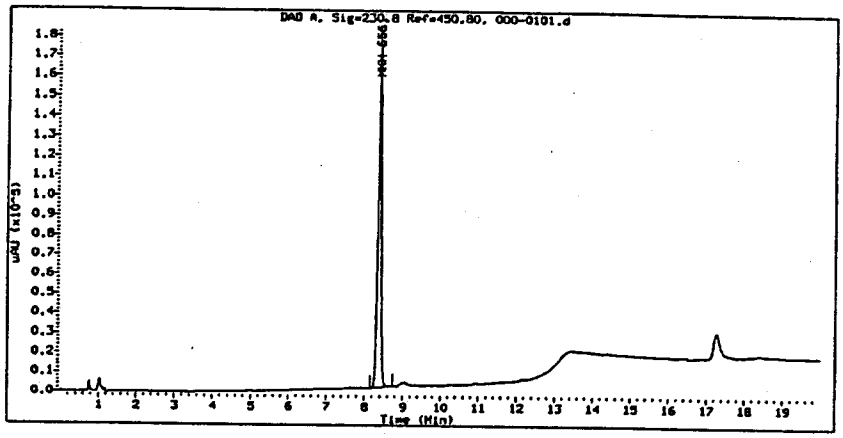
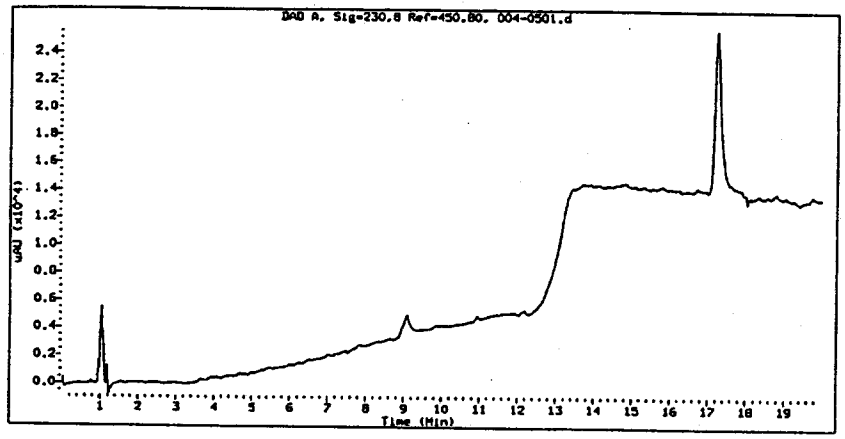


Figure 2.2: typical LC-chromatograms of rodent ration samples:
test material concentrations: 0 ppm (untreated control sample) and 1000 ppm



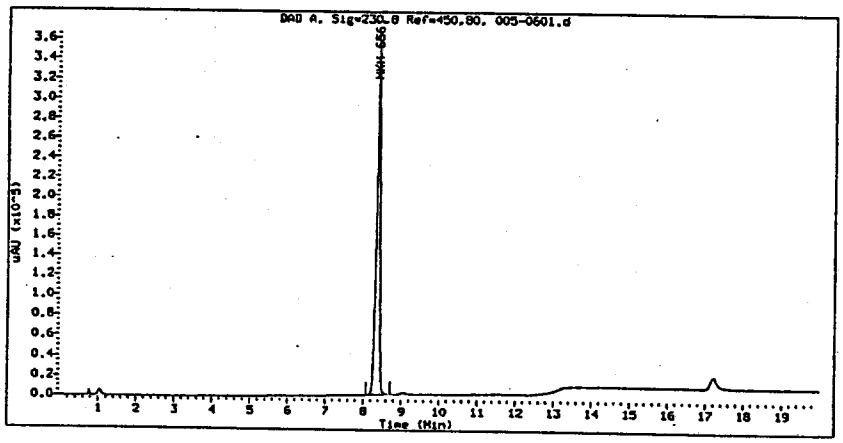
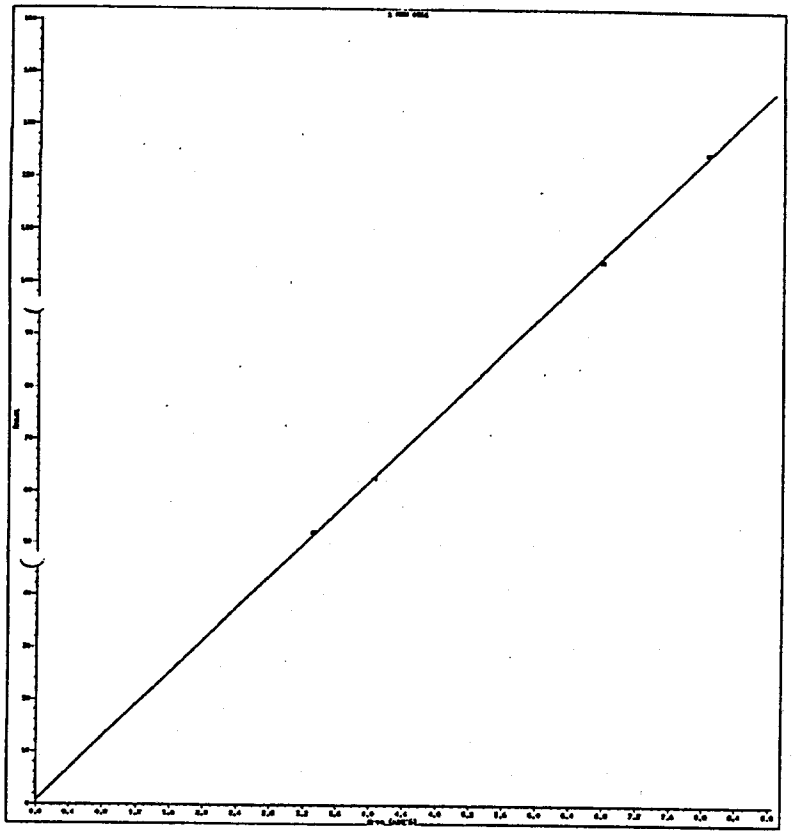


Figure 3: Calibration curve of the analytical method (Date: Sep. 2, 1996)



The calibration was linear in the range shown. The linear regression value was $r^2 = 1.000$

9. PRECISION

The precision of this analytical method was assessed by 10 separate injections for two relevant concentrations of the calibration standards. The concentration values obtained with a statistical evaluation (coefficient of variation = %RSD) were presented in Table 1 (data from [3]). The precision of this method was found to satisfy the analytical requirements.

Table 1:

20.490 [µg/ml]	204.900 [µg/ml]
20.071	204.245
20.225	200.602
20.057	200.540
20.159	200.519
20.191	199.165
19.837	200.241
20.123	196.963
20.216	199.163
19.330	199.352
20.060	200.854
MEAN = 20.027	**MEAN = 200.164**
%RSD = 1.3%	**%RSD = 0.9%**

10. RECOVERY

The recoveries from rodent ration were evaluated in fortification tests. Known amounts of test compound were added to untreated control feed - Altromin 1321 - prior to extraction.
Date of preparation: Jan. 22, 1995 ; (data presented in study No. T0058151)

Result:

The analytical data verify that the test material recovery was assured within the concentration range of 250 to 20000 ppm (= 93.9%).

Table 2:

active ingredient added [ppm]	sample	actual-concentration [%] from target	mean value [% ; %RSD]
250 ppm	prep.1 -- 1st inj.	97.40	
250 ppm	prep.1 -- 2nd inj.	92.04	
250 ppm	prep.2 -- 1st inj.	98.72	
250 ppm	prep.2 -- 2nd inj.	99.59	
250 ppm	prep.3 -- 1st inj.	104.60	
250 ppm	prep.3 -- 2nd inj.	99.61	
250 ppm	prep.4 -- 1st inj.	97.54	
250 ppm	prep.4 -- 2nd inj.	95.17	
250 ppm	prep.5 -- 1st inj.	94.36	
250 ppm	prep.5 -- 2nd inj.	92.51	**97.15%** ; {3.9%}
20000 ppm	prep.1 -- 1st inj.	89.53	
20000 ppm	prep.1 -- 2nd inj.	88.47	
20000 ppm	prep.2 -- 1st inj.	94.21	
20000 ppm	prep.2 -- 2nd inj.	93.67	
20000 ppm	prep.3 -- 1st inj.	92.59	
20000 ppm	prep.3 -- 2nd inj.	92.41	
20000 ppm	prep.4 -- 1st inj.	88.66	
20000 ppm	prep.4 -- 2nd inj.	89.13	
20000 ppm	prep.5 -- 1st inj.	89.87	
20000 ppm	prep.5 -- 2nd inj.	88.82	**90.74%** ; { 2.5%}

11. ANALYTICAL RESULTS

The analytical data verify that the test material was homogeneously distributed (Tab. 3). and chemically stable within the concentration range of 100 ppm to 20000 ppm (Tab. 4). Under current sample preparation and handling conditions stability in the diet was assured for a period of at least 14 days.

For stability testing the samples were stored under conditions comparable to those in the actual study and then frozen until analytical measurement.
These analytical raw data (homogeneity, stability) were presented by D.I.Riegner, PF-E/MR in the study No. T2055651 [2].

11.1. HOMOGENEITY

Tables 3.1-3.2 present the analytical results from the three samples collected from a high and low target concentration of feed mixtures.

Table 3.1: **Homogeneity** tests (diet batch No. 1211); Weight of mixture: 6 kg
date of preparation: March 29, 1994

	target concentration	
	100 ppm	20000 ppm
Mean:	101 ppm	19300 ppm
%RSD:	2.6%	0.5%

Table 3.2: **Homogeneity** tests (diet batch No. 1526); Weight of mixture: 6 kg
date of preparation: March 29, 1994

	target concentration	
	100 ppm	20000 ppm
Mean:	103 ppm	19300 ppm
%RSD:	2.6%	0.8%

11.2. STABILITY

Table 4 presents analytical results from sequential evaluations of the two test mixture concentrations. The calculation [%] of target concentrations based on the analytical result on day 0!

Table 4: **Stability** (in [%] of target concentration and actual weight units [ppm])
date of preparation {I}

sample	100 ppm Lot No. 1211	20000 ppm Lot No. 1211	100 ppm Lot No. 1526	20000 ppm Lot No. 1526
I: March 29, 1994 (stab. test-start)	101% (101 ppm)	97% (19300 ppm)	103% (103 ppm)	97% (19300 ppm)
I: March 29, 1994 (stab. 14 days)	97% (97.5 ppm) based on 0 h	101% (19400 ppm) based on 0 h	102% (105 ppm) based on 0 h	103% (19800 ppm) based on 0 h

11.3. CONTENT CHECK FOR DOSE VERIFICATION

The analytical data (HPLC) verify that the test material content in the toxicology test mixtures agreed with the target concentrations within defined limits (Tab. 5). Additionally a stability test of these samples was performed. The samples were stored under conditions comparable to those in the actual study, and then quantified.

For calculations integrator values from each sample were based on the external standard calibration curve of the active ingredient. The analytical results of the test material were expressed in weight units [ppm]. For assessment of content checks the percentage of active ingredient in the original test material was not included for calculations.

Table 5 presents analytical results from sequential evaluations of the animal rations concentrations. The calculation [%] of target concentration was based on the analytical result on day 0! In analyzed control samples amounts of active ingredient were not detected.

Table 5: Content (in [%] of target concentration and actual weight units [ppm]) date of preparation {I} / freezing {II} / samples thawed and date of measurement: {III}

sample	1000 ppm	5000 ppm	20000 ppm
I+II: July 3, 1996 (stab. test-start)	109.4% (1094.33 ppm)	108.5% (5422.68 ppm)	109.8% (21962.51 ppm)
I: July 3, 1996 II: July 10, 1996 III: July 16, 1996 (stab. 7 days)	99.9% (1092.65 ppm) based on day 0	101.4% (5498.40 ppm) based on day 0	97.9% (21510.89 ppm) based on day 0
I+II: Aug. 21, 1996 (stab. test-start)	109.3% (1092.68 ppm)	109.7% (5486.42 ppm)	113.3% (22658.69 ppm)
I: Aug. 21, 1996 II: Aug. 28, 1996 III: Sep. 2, 1996 (stab. 7 days)	102.5% (1119.62 ppm) based on day 0	103.5% (5677.78 ppm) based on day 0	100.0% (22659.52 ppm) based on day 0
I+II: Sep. 25, 1996 (stab. test-start)	105.0% (1049.9 ppm)	106.3% (5312.7 ppm)	106.6% (21311.2 ppm)
I: Sep. 25, 1996 III: Oct. 2, 1996 (stab. 7 days)	102.0% (1070.4 ppm) based on day 0	98.7% (5244.4 ppm) based on day 0	100.2% (21352.4 ppm) based on day 0

12. LITERATURE

[1] D.I.Riegner, PF-E/MR, report No. MR-0556/94; Nov. 9, 1994
Summary of Analytical Data on MKH 6561 (study No. T0058151)

[2] D.I.Riegner, PF-E/MR, report No. RA-0359/94; Sep. 22, 1994
Summary of Analytical Data on MKH 6561 (study No. T2055651)

[3] Dr. W. Ruengeler, PD-T Tox. Analytic,
Analytical Part of the study report MKH 6561 (study No. T7060911)

End of Report

NUTRIENT COMPOSITION OF DIET FOR RAT / MOUSE (Altromin 1321/1324)

Ingredients *

Crude protein	19.0
Crude fat	4.0
Crude fiber	6.0
Ash	7.0
Moisture	13.5
Nitrogen-free extract	50.5
Metabolizable energy:	
Kcal / kg	2850.0
KJ / kg	11900.0

Minerals *

Calcium	0.9
Phosphorus	0.7
Magnesium	0.2
Sodium	0.2
Potassium	1.0

Vitamins *** Standard-Diet

Vitamin A	15000.0 IU
Vitamin D3	600.0 IU
Vitamin E	75.0 mg
Vitamin K3	3.0 mg
Vitamin B1	18.0 mg
Vitamin B2	12.0 mg
Vitamin B6	9.0 mg
Vitamin B12	24.0 mcg
Nicotinic acid	36.0 mg
Pantothenic acid	21.0 mg
Folic acid	2.0 mg
Biotin	60.0 mcg
Choline	600.0 mg
Vitamin C	36.0 mg

Amino acids *

Lysine	0.90
Methionine	0.30
Cystine	0.30
Phenylalanine	0.80
Tyrosine	0.60
Arginine	1.10
Histidine	0.40
Tryptophane	0.20
Threonine	0.60
Isoleucine	0.80
Leucine	1.30
Valine	0.90

Trace elements **

Manganese	75.0
Iron	180.0
Copper	13.0
Zinc	70.0
Iodine	0.9
Fluorine	15.0

* Average % content in the diet
** Average mg content in 1 kg diet
*** Additive/1 kg diet

valid from 1987 onwards

CONTAMINANTS IN DIET FOR RAT / MOUSE (Altromin 1321/1324)

Contaminant	Detection Limit		Maximun Content
Mycotoxins			
Aflatoxin			
B1	2 ppb		10 ppb
B2	2 ppb		5 ppb
G1	2 ppb		5 ppb
G2	2 ppb		5 ppb
Organo-Cl-Compounds			
Tecnazene	0.001 mg/kg		not fixed
HCB (Hexachlorbenzene)	0.001 mg/kg		0.01 mg/kg
alpha-HCH	0.001 mg/kg		0.02 mg/kg
beta-HCH	0.001 mg/kg		0.02 mg/kg
gamma-HCH (Lindane)	0.001 mg/kg		0.10 mg/kg
delta-HCH	0.001 mg/kg		0.02 mg/kg
Quintozene	0.001 mg/kg		0.01 mg/kg
Heptachlor	0.001 mg/kg	as)	0.01 mg/kg
Heptachlorepoxide	0.003 mg/kg	Heptachlor)	
alpha-Chlordane	0.005 mg/kg		0.02 mg/kg
gamma-Chlordane	0.005 mg/kg		0.02 mg/kg
alpha-Endosulphane	0.005 mg/kg		0.10 mg/kg
beta-Endosulphane	0.005 mg/kg		0.10 mg/kg
Aldrin	0.003 mg/kg	as)	0.01 mg/kg
Dieldrin	0.003 mg/kg	Dieldrin)	
Endrin	0.003 mg/kg		0.01 mg/kg
o,p-DDE	0.002 mg/kg	)	
p,p-DDE	0.002 mg/kg	)	
o,p-DDD	0.002 mg/kg	as)	0.05 mg/kg
o,p-DDT	0.002 mg/kg	D D T)	
p,p-DDD	0.002 mg/kg	)	
p,p-DDT	0.002 mg/kg	)	
Methoxychlor	0.01 mg/kg		not fixed

valid from 1987 onwards

CONTAMINANTS IN DIET FOR RAT/MOUSE (Altromin 1321/324)

Contaminant	Detection Limit	Maximun Content
Organo-P-Compounds		
Chlorthion	0.01 mg/kg	0.5 mg/kg
Disulfoton	0.005 mg/kg	0.5 mg/kg
Malathion	0.01 mg/kg	1.0 mg/kg
Parathion (-methyl)	0.005 mg/kg	0.5 mg/kg
Parathion (-ethyl)	0.01 mg/kg	0.5 mg/kg
Sulfotep	0.002 mg/kg	0.5 mg/kg
Fenthion	0.005 mg/kg	1.0 mg/kg
Dimethoate	0.005 mg/kg	1.0 mg/kg
Trichlorphon	0.01 mg/kg	1.0 mg/kg
Fenitrothion	0.01 mg/kg	1.0 mg/kg
Bromophos (-methyl)	0.01 mg/kg	1.0 mg/kg
Bromophos (-ethyl)	0.01 mg/kg	1.0 mg/kg
Chlorfenvinphos	0.01 mg/kg	0.5 mg/kg
Pirimiphos (-methyl)	0.01 mg/kg	1.0 mg/kg
Methidathion	0.01 mg/kg	1.0 mg/kg
Ethion	0.01 mg/kg	0.5 mg/kg
Heavy Metals		
Lead	0.1 mg/kg	1.5 mg/kg
Cadmium	0.01 mg/kg	0.4 mg/kg
Mercury	0.01 mg/kg	0.1 mg/kg
Arsenic	0.2 mg/kg	1.0 mg/kg
Selenium	0.1 mg/kg	0.6 mg/kg
Copper	1.0 mg/kg	not fixed
PCB's	0.01 mg/kg	0.05 mg/kg

Tolerance ranges of analysis:

Detection Limit	Tolerance
5 - 100 ppb	+/- 50 % relative
100 - 200 ppb	+/- 50 ppb absolute
above 200 ppb	+/- 25 % relative

valid from 1987 onwards

SPECIFICATION OF TAP WATER

(according to "Trinkwasser-Verordnung " 12-05-90, BGBL No. 66 edited 12-12-90, page 2612-2629)

Limits of Chemical Substances in Tap-Water

Substance	Limit mg/l	corresponding to approx. mmol/m³	calculated as
Arsenic	0.04 *	0.5	As
Lead	0.04	0.2	Pb
Cadmium	0.005	0.04	Cd
Chromium	0.05	1	Cr
Cyanide	0.05	2	CN (-)
Fluoride	1.5	79	F (-)
Nickel	0.05	0.9	Ni
Nitrate	50	806	NO_3 (-)
Nitrite	0.1	2.2	NO_2 (-)
Mercury	0.001	0.005	Hg
PAH **	0.0002	0.02	C
Organic Chloride Compounds ***			
- 1,1,1-Trichloroethane Trichloroethene Tetrachloroethene Dichloromethane	0.025		
- Tetrachloromethane	0.003	0.02	CCl_4
Pesticides and similar compounds			
- per compound	0.0001		
- compounds in total	0.0005		

* from January 1, 1996: 0.01 mg/l

** PAH = Polycyclic Aromatic Hydrocarbons

*** from January 1, 1992: Compounds in total 0.01 mg/l
Tetrachloromethane 0.003 mg/l

valid from 1991 onwards

Parameters and limits for the evaluation of the quality of drinking water
(appendix 4 of the "Trinkwasserverordnung")

I. SENSORY PARAMETERS

	Factor	Limit
1	Coloration	0.5 m^{-1}
2	Turbidity	1.5 turbidity units / formazine
3	Odour threshold	2 at 12 °C 3 at 25 °C

II. PHYSICOCHEMICAL PARAMETERS

	Parameters	Limit	calculated as
4	Temperature	25 °C	
5	pH	not less than 6.5 not more than 9.5	
6	Conductivity	2000 µ S cm^{-1} at 25 °C	
7	Oxidizability	5 mg/l	O_2

III. LIMITS FOR CHEMICAL SUBSTANCES

	Parameters	Limit mg/l	calculated as	corresponding to approx. mmol/m^3
8	Aluminium	0.2	Al	7.5
9	Ammonium	0.5	NH_4^+	30
10	Iron	0.2	Fe	3.5
11	Potassium	12	K	300
12	Magnesium	50	Mg	2050
13	Manganese	0.05	Mn	0.9
14	Sodium	150	Na	6500
15	Silver	0.01	Ag	0.1
16	Sulphate	240	SO_4^{2-}	2500
17	Surfactants			
	a) anionic	0.2	a) Methyleneblue active substances	
	b) non-ionic	0.2	b) Bismuth active substances	

valid from 1991 onwards

IV. MICROBIOLOGICAL PARAMETERS

Parameters	Volume of sample to be investigated	Maximal tolerated germ titer
Coliforms	100 ml	0
E.coli	100 ml	0
Streptococcus fecalis	100 ml	0
Sulphite reducing clostridium	20 ml	0

Total number of colonies in 1 ml drinking water should not exceed 100

(incubation temperature 20 ± 2 °C and 36 ± 1 °C

Calculation of FEED CONSUMPTION and ACTIVE INGREDIENT INTAKE

Body weights and the initial and final weights of diet are measured in grams for the calculation.

1 FEED CONSUMPTION

1.1 Feed Consumption per Animal per Day

$$= \frac{H - R}{nT}$$

H = Weight of administered feed (if necessary, plus weight of feed container) at time of weighing (initial weight)

R = Weight of unconsumed feed (if necessary, plus weight of feed container) at time of weighing back (final weight)

nT = Number of days between weighing and weighing back

1.2 Mean Feed Consumption per Animal per Day (Date-Related)

$$= \frac{\text{Sum of All Values Available at a Specific Date}}{\text{No. of Values}}$$

All feed consumption values existing at a specific date (per animal per day, see 1.1) are totaled up. This total is divided by the number of values existing at that date.

1.3 Mean Feed Consumption per Animal per Day

$$= \frac{\text{Sum of All Values}}{\text{No. of Values}}$$

All existing feed consumption values (per animal per day, see 1.1) are totaled up. This total is divided by the number of existing values.

1.4 Cumulative Feed Consumption per Animal

= (Mean Feed Consumption per Animal per Day) x nDays

For mean feed consumption per animal per day, see 1.3. nDays is established from the total number of feed consumption days.

1.5 Food Consumption per kg Body Weight per Day

$$= \frac{\text{Feed Consumption per Animal per Day}}{\text{Body Weight of the Animal}} \times 1000$$

For food consumption per animal per day, see 1.1. The value that was obtained within the time interval from the day of weighing back (final wt.) to the day of weighing back minus 7 is taken as the basis for the body weight. If no determination of the body weight of the animals within his time interval was planned, the time interval from the day of weighing back to the day of weighing back plus 6 is taken as the basis. If no body weight value is available within either of these two time intervals, no food consumption is calculated.

1.6 Mean Food Consumption per kg Body Weight per day

$$= \frac{\text{Sum of all Values Available at a Specific Date}}{\text{No. of Values}}$$

All food consumption values existing at a specific date (per kg body weight per day, see 1.5) are totaled up. This total is divided by the number of values existing at that date.

1.7 Mean Food Consumption per kg Body Weight per Day

$$= \frac{\text{Sum of All Values}}{\text{No. of Values}}$$

All existing food consumption values (per kg body weight per day, see 1.5) are totaled up. This total is devided by the number of existing values.

1.8 Cumulative Food Consumption per kg Body Weight

= (Mean Food Consumption per kg Body Weight per Day) x nDays

For mean food consumption per kg body weight per day,see 1.7. nDays is established from the total number of food consumption days.

2. ACTIVE INGREDIENT (AI) INTAKE

The active ingredient (AI) intake is calculated from the food consumption data by using a "Dose Factor".
where: Dose in ppm, Food consumption in g, AI intake in mg

$$\text{Dose Factor} = \frac{\text{Dose}}{1000}$$

2.1 Mean AI Intake per Animal per Day

= (Mean Food Consumption per Animal per Day) x Dose Factor
For mean food consumption per animal per day (see 1.3).

2.2 Cumulative AI Intake per Animal

= (Cumulative Food Consumption per Animal) x Dose Factor
For cumulative food consumption per animal (see 1.4).

2.3 AI Intake per kg Body Weight per Day

= (Food Consumption per kg Body Weight per Day) x Dose Factor
For food consumption per kg body weight per day (see 1.5).

2.4 Mean AI Intake per kg Body Weight per Day

= (Mean Food Consumption per kg Body Weight per Day at a Specific Date) x Dose Factor
For mean food consumption per kg body weight per day at a specific date (see 1.6).

2.5 Mean AI Intake per kg Body Weight per Day

= (Mean Food Consumption per kg Body Weight per Day) x Dose Factor
For mean food consumption per kg body weight per day (see 1.7).

2.6 Cumulative AI Intake per kg Body Weight

= (Cumulative Food Consumption per kg Body Weight) x Dose Factor.For cumulative food consumption per kg body weight (see 1.8.).

U e b e r l e b e n d e T i e r e / s u r v i v i n g a n i m a l s

Dosis/ dose (PPM)	Sex sex	Appl adm	n		Woche / week 0	1	2	3	4	5
0	m m	PO	10	L	10	10	10	10	10	10
1000	m m	PO	10	L	10	10	10	10	10	10
5000	m m	PO	10	L	10	10	10	10	10	10
20000	m m	PO	10	L	10	10	10	10	10	10

n = Anzahl eingesetzter Tiere/ number of animals used
L = Anzahl ueberlebender Tiere (Ende der Woche)/number of surviving animals (end of week)

U e b e r l e b e n d e T i e r e / s u r v i v i n g a n i m a l s

Dosis/ dose (PPM)	Sex sex	Appl adm	n		Woche / week 0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	w f	PO	10	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10
1000	w f	PO	10	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5000	w f	PO	10	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10
20000	w f	PO	10	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10

n = Anzahl eingesetzter Tiere/ number of animals used
L = Anzahl ueberlebender Tiere (Ende der Woche)/number of surviving animals (end of week)

Ueberlebende Tiere / surviving animals

Dosis/ dose (PPM)	Sex sex	Appl adm	n		Woche / week 14
0	w	PO	10	L	10
	f				
1000	w	PO	10	L	10
	f				
5000	w	PO	10	L	10
	f				
20000	w	PO	10	L	10
	f				

n = Anzahl eingesetzter Tiere/ number of animals used
L = Anzahl ueberlebender Tiere (Ende der Woche)/number of surviving animals (end of week)

i n d i v i d u a l c l i n i c a l f i n d i n g s

all findings

from week 0 to 15

anim. no.	finding	week
F0 Generation 0 PPM female PO		
17	I EYE BLOODY I RIGHT I	11; 11
F0 Generation 5000 PPM female PO		
60	I EYE BLOODY I RIGHT	10 - 11; 11; 12

Scheme of Mating of the F0 Generation

Male No. Mated with Female No.							
0 ppm		1000 ppm		5000 ppm		20000 ppm	
Male	Female	Male	Female	Male	Female	Male	Female
1	17	21	37	41	56	61	78
2	16	22	38	42	53	62	74
3	13	23	31	43	54	63	72
4	19	24	36	44	55	64	80
5	18	25	40	45	58	65	77
6	11	26	33	46	59	66	73
7	12	27	39	47	52	67	76
8	14	28	35	48	57	68	79
9	15	29	34	49	51	69	71
10	20	30	32	50	60	70	75

Body Weights

(g)

012334/97.001

	Week													
	0	1	2	3	4	6	7	8	9	10	11	11	12	13
F0 Generation 0 PPM Male PO														
Mean	311	338	356	372	386									
Med.	311	333	350	366	383									
S.D.	23.0	27.0	28.0	29.6	29.7									
Min.	284	310	328	337	355									
Max.	344	386	409	434	451									
N	10	10	10	10	10									
F0 Generation 1000 PPM Male PO														
Mean	301	329	349	361	374									
Med.	292	323	340	353	367									
S.D.	22.6	25.4	28.8	28.0	29.3									
Min.	272	292	315	324	341									
Max.	337	370	401	410	432									
N	10	10	10	10	10									
TS 1%	-	-	-	-	-									
TS 5%	-	-	-	-	-									
F0 Generation 5000 PPM Male PO														
Mean	299	330	351	369	378									
Med.	294	324	345	366	375									
S.D.	22.8	26.1	31.8	34.3	37.0									
Min.	265	294	311	327	337									
Max.	345	380	418	437	454									
N	10	10	10	10	10									
TS 1%	-	-	-	-	-									
TS 5%	-	-	-	-	-									
F0 Generation 20000 PPM Male PO														
Mean	304	325	351	366	378									
Med.	304	320	346	360	373									
S.D.	23.6	22.3	23.1	24.2	24.1									
Min.	266	294	319	326	336									
Max.	347	370	396	413	421									
N	10	10	10	10	10									
TS 1%	-	-	-	-	-									
TS 5%	-	-	-	-	-									

Body Weights

(g)

Pregnancy Lactation 115434/97.002

	Week													
	0	1	2	3	4	6	7	8	9	10	10	11	12	13
F0 Generation 0 ppm Female P0														
Mean	184	190	196	203	205	200	229	241	280	243	255	262	226	255
Med.	183	190	197	200	202	203	229	241	288	243	257	263	220	253
S.D.	12.9	14.1	15.8	17.0	16.7	17.0	17.4	23.6	51.3	17.7	21.9	19.2	21.0	15.6
Min.	168	169	169	174	184	174	210	209	214	213	228	236	202	237
Max.	206	209	216	233	228	223	255	277	346	267	283	288	260	281
N	10	10	10	10	10	9	9	9	9	8	8	8	8	8
F0 Generation 1000 ppm Female P0														
Mean	185	194	200	208	210	204	233	252	307	234	250	259	228	252
Med.	187	197	204	213	213	205	235	248	311	232	244	257	226	258
S.D.	14.4	12.6	14.5	14.6	15.6	17.3	20.3	24.9	44.1	22.7	20.5	18.3	17.7	22.6
Min.	164	177	177	181	185	183	208	224	226	202	230	236	201	222
Max.	207	212	217	227	232	231	268	298	371	278	286	292	256	282
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0 Generation 5000 ppm Female P0														
Mean	180	191	200	205	207	206	233	251	304	229	246	257	223	250
Med.	181	194	203	202	207	204	231	249	318	232	248	257	217	255
S.D.	8.53	11.1	11.8	11.1	11.5	13.3	11.7	18.6	42.0	22.0	14.2	13.8	23.7	19.0
Min.	168	175	182	191	187	188	217	217	225	198	227	237	194	212
Max.	193	209	219	224	223	229	254	281	343	268	272	281	279	272
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0 Generation 20000 ppm Female P0														
Mean	184	192	200	206	211	204	234	245	278	243	256	264	238	263
Med.	185	191	201	206	211	204	233	236	255	245	257	265	237	273
S.D.	11.3	12.1	14.6	14.1	15.5	12.9	14.4	20.3	44.6	17.0	17.1	21.7	35.9	19.1
Min.	158	167	168	175	179	184	207	228	239	206	220	221	192	232
Max.	200	211	226	233	241	222	255	283	349	266	277	294	288	281
N	10	10	10	10	10	9	9	9	9	8	8	8	7	7
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Body Weight Gain

(g)

012354/97.001

	Week			
	0	1	2	3
	1	2	3	4

F0 Generation 0 PPM Male P0

Mean	26.6	18.3	15.3	14.3
Med.	26.9	18.0	16.0	13.4
S.D.	8.03	4.46	4.85	4.94
Min.	15.2	10.5	8.10	8.00
Max.	41.8	24.5	24.1	22.3
N	10	10	10	10

F0 Generation 1000 PPM Male P0

Mean	27.8	20.0	12.6	13.3
Med.	27.7	20.1	12.6	14.8
S.D.	6.35	8.94	3.14	6.21
Min.	17.3	6.70	8.80	3.90
Max.	35.4	36.5	17.4	21.8
N	10	10	10	10
TS 1%	-	-	-	-
TS 5%	-	-	-	-

F0 Generation 5000 PPM Male P0

Mean	30.5	21.2	17.9	9.92
Med.	29.3	21.0	18.7	11.1
S.D.	4.39	7.45	4.53	4.87
Min.	26.5	11.0	10.1	2.30
Max.	39.8	38.2	26.2	17.0
N	10	10	10	10
TS 1%	-	-	-	-
TS 5%	-	-	-	-

F0 Generation 20000 PPM Male P0

Mean	21.1	26.5	14.5	11.9
Med.	21.1	25.4	14.6	11.4
S.D.	14.3	6.53	4.93	2.86
Min.	-13	18.6	7.60	8.70
Max.	38.3	43.2	22.2	15.9
N	10	10	10	10
TS 1%	-	-	-	-
TS 5%	-	+	-	-

Body Weight Gain

(g)

115454/97.002

	Week												
	0	1	2	3	4	6	7	8	9	10	11	11	12
	1	2	3	4	6	7	8	9	10	11	11	12	13
F0 Generation 0 ppm Female P0													
Mean	5.45	5.85	7.06	2.33	-3.7	29.2	11.7	38.6	-52	12.2	7.25	-37	29.8
Med.	5.10	6.25	5.60	1.90	-4.3	31.0	18.0	60.0	-63	13.5	9.00	-42	27.0
S.D.	4.71	4.83	5.01	5.27	9.71	5.85	13.6	31.5	45.8	10.6	6.76	22.8	23.6
Min.	-1.7	-2.3	0.40	-5.0	-17	19.0	-7.0	-1.0	-102	-8.0	-5.0	-69	-7.0
Max.	12.8	12.4	17.0	9.80	16.9	36.0	30.0	71.0	13.0	24.0	17.0	8.00	72.0
N	10	10	10	10	9	9	9	9	7	8	8	8	8
F0 Generation 1000 ppm Female P0													
Mean	8.91	5.45	8.43	1.95	-5.6	29.1	18.8	54.7	-73	16.2	9.40	-31	23.5
Med.	9.15	4.50	9.30	1.70	-4.9	28.5	21.0	63.5	-87	15.5	8.00	-34	27.0
S.D.	3.36	4.04	3.07	5.05	5.30	5.51	9.80	24.4	34.4	8.32	6.47	16.9	19.7
Min.	3.20	-.40	3.40	-4.5	-15	19.0	-2.0	2.00	-108	5.00	2.00	-51	-4.0
Max.	13.7	11.0	11.9	11.2	1.90	37.0	30.0	77.0	-9.0	32.0	23.0	11.0	58.0
N	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-
F0 Generation 5000 ppm Female P0													
Mean	11.1	8.33	4.95	2.45	-1.4	26.9	18.5	52.4	-75	16.9	11.2	-34	26.9
Med.	9.90	7.85	5.70	3.85	-2.4	27.0	21.0	63.5	-99	16.0	11.0	-43	35.5
S.D.	4.11	2.52	3.95	3.98	9.74	4.09	11.1	26.7	48.4	11.1	4.61	26.8	20.0
Min.	6.40	5.00	-3.1	-3.5	-18	21.0	-1.0	-2.0	-119	4.00	1.00	-70	-7.0
Max.	19.8	13.2	9.70	7.00	17.6	36.0	31.0	71.0	14.0	36.0	19.0	25.0	49.0
N	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	++	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	+	-	-	-	-	-	-	-	-	-	-	-	-
F0 Generation 20000 ppm Female P0													
Mean	8.80	8.06	6.00	4.56	-3.3	29.9	11.1	32.7	-48	13.4	7.75	-26	25.0
Med.	9.50	8.80	5.55	4.30	-1.7	32.0	7.00	22.0	-46	12.5	7.50	-29	7.00
S.D.	3.26	6.21	2.12	3.96	7.44	9.68	14.9	26.1	50.5	4.21	5.63	27.0	30.0
Min.	1.50	0.70	3.10	-1.4	-18	13.0	-11	5.00	-104	8.00	0.00	-57	-7.0
Max.	11.9	15.2	10.1	10.0	6.30	42.0	29.0	73.0	12.0	21.0	17.0	14.0	70.0
N	10	10	10	10	9	9	9	9	7	8	8	7	7
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-

Food Consumption

(g/d)

012344/97.001

	Week 1	2	3	4
F0 Generation 0 PPM Male PO				
Mean	20.4	20.6	21.5	21.5
Med.	20.0	20.5	21.3	21.5
S.D.	1.90	1.71	1.96	2.15
Min.	17.2	18.5	18.7	16.8
Max.	23.5	23.9	24.6	24.4
N	10	10	10	10
F0 Generation 1000 PPM Male PO				
Mean	20.9	19.7	22.7	21.9
Med.	20.4	19.5	22.2	21.5
S.D.	2.14	2.25	2.35	1.25
Min.	18.5	16.8	19.2	20.4
Max.	26.1	23.5	27.0	23.6
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-
F0 Generation 5000 PPM Male PO				
Mean	19.7	20.3	24.1	21.2
Med.	19.5	20.1	22.9	20.3
S.D.	.964	1.97	4.62	2.99
Min.	18.0	17.1	20.8	18.2
Max.	21.3	23.3	36.2	26.4
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-
F0 Generation 20000 PPM Male PO				
Mean	19.4	21.4	23.6	22.8
Med.	19.3	21.7	23.3	22.4
S.D.	1.04	1.29	1.99	1.77
Min.	18.1	19.7	21.0	20.8
Max.	21.1	23.2	27.2	25.8
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-

Food Consumption

(g/d)

012344/97.002

	Week 1	2	3	4
F0 Generation 0 PPM Female PO				
Mean	14.4	13.3	21.0	16.4
Med.	14.4	12.8	17.0	14.5
S.D.	2.06	2.27	8.86	5.41
Min.	11.9	10.7	14.1	11.9
Max.	17.9	18.5	42.9	31.0
N	10	10	10	10
F0 Generation 1000 PPM Female PO				
Mean	13.3	13.0	15.9	14.1
Med.	13.0	12.9	16.1	14.2
S.D.	1.08	1.27	2.27	1.69
Min.	11.8	11.4	12.2	11.5
Max.	15.0	15.3	19.5	16.6
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-
F0 Generation 5000 PPM Female PO				
Mean	15.0	14.1	17.8	14.7
Med.	13.9	14.5	17.3	15.0
S.D.	4.37	1.41	4.11	1.55
Min.	11.7	11.4	12.3	12.2
Max.	26.8	16.3	25.8	17.4
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-
F0 Generation 20000 PPM Female PO				
Mean	14.9	14.2	17.4	16.1
Med.	14.0	14.4	16.7	15.3
S.D.	2.73	1.36	3.40	2.18
Min.	12.1	12.4	13.8	14.1
Max.	19.1	16.3	26.1	21.0
N	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-

Absolute Organ Weights

013564/97.001

	Body W.	Brain	Liver	Kidneys	Testes
	G	mg	mg	mg	mg
FO Generation 0 PPM Male PO					
Mean	448	1959	15135	2594	3427
Med.	445	1951	14863	2660	3480
S.D.	26.9	61.7	1502.3	200.9	230.8
Min.	419	1886	12911	2214	2870
Max.	507	2056	17688	2874	3701
N	10	10	10	10	10
FO Generation 1000 PPM Male PO					
Mean	442	1994	15219	2464	3371
Med.	429	1979	14991	2445	3405
S.D.	40.0	74.4	1555.1	135.0	230.6
Min.	404	1922	13142	2273	2949
Max.	527	2154	18009	2660	3683
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-
FO Generation 5000 PPM Male PO					
Mean	446	1937	15386	2370	3430
Med.	447	1922	15775	2305	3386
S.D.	34.7	60.9	1339.5	210.0	454.3
Min.	399	1862	12919	2081	2953
Max.	503	2037	17096	2692	4393
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	+	-
FO Generation 20000 PPM Male PO					
Mean	444	1938	14242	2502	3530
Med.	442	1944	14251	2574	3590
S.D.	26.1	99.3	1274.1	219.4	264.5
Min.	396	1766	12362	2171	3103
Max.	475	2055	16056	2811	3931
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-

Absolute Organ Weights

013564/97.002

	Body W.	Brain	Liver	Kidneys	Ovaries
	G	mg	mg	mg	mg
FO Generation 0 PPM Female PO					
Mean	254	1777	11317	1595	112
Med.	251	1764	10627	1577	97
S.D.	18.5	63.5	2793.4	131.8	28.6
Min.	237	1704	7806	1335	83
Max.	292	1912	16691	1853	152
N	10	10	10	10	10
FO Generation 1000 PPM Female PO					
Mean	258	1741	13120	1685	104
Med.	259	1748	12633	1696	104
S.D.	19.8	57.3	1401.0	110.5	23.6
Min.	225	1657	11087	1504	74
Max.	282	1817	15234	1857	149
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-
FO Generation 5000 PPM Female PO					
Mean	254	1719	12675	1802	103
Med.	255	1727	12733	1774	102
S.D.	16.5	46.5	2093.6	117.2	23.7
Min.	213	1645	8968	1630	56
Max.	273	1798	16724	2018	129
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	+	-
FO Generation 20000 PPM Female PO					
Mean	265	1753	11590	1724	123
Med.	266	1788	12462	1736	119
S.D.	15.5	97.4	2926.1	263.2	17.1
Min.	234	1604	7527	1272	103
Max.	286	1874	15487	2142	150
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-

Relative Organ Weights

Relative to Body Weights

015754/97.001

	Body W.	Brain	Liver	Kidneys	Testes
	G	mg/100g	mg/100g	mg/100g	mg/100g
F0 Generation 0 PPM Male PO					
Mean	448	438	3380	579	767
Med.	445	444	3409	574	787
S.D.	26.9	23.4	280.2	38.1	60.0
Min.	419	394	2914	516	644
Max.	507	475	3883	639	825
N	10	10	10	10	10
F0 Generation 1000 PPM Male PO					
Mean	442	453	3442	560	764
Med.	429	465	3456	565	768
S.D.	40.0	32.2	214.1	51.9	50.7
Min.	404	408	3162	472	699
Max.	527	487	3907	649	840
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-
F0 Generation 5000 PPM Male PO					
Mean	446	436	3450	532	773
Med.	447	434	3473	530	743
S.D.	34.7	33.4	183.9	42.8	118.8
Min.	399	392	3177	450	618
Max.	503	505	3738	590	969
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	+	-
F0 Generation 20000 PPM Male PO					
Mean	444	437	3209	563	798
Med.	442	435	3239	563	812
S.D.	26.1	19.0	187.3	28.1	69.7
Min.	396	405	2865	516	687
Max.	475	466	3412	599	894
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-

Relative Organ Weights

Relative to Body Weights

015754/97.002

	Body W.	Brain	Liver	Kidneys	Ovaries
	G	mg/100g	mg/100g	mg/100g	mg/100g
FO Generation 0 PPM Female PO					
Mean	254	701	4412	628	44
Med.	251	704	4330	639	39
S.D.	18.5	42.7	825.1	43.7	12.6
Min.	237	635	3187	545	29
Max.	292	770	5921	676	62
N	10	10	10	10	10
FO Generation 1000 PPM Female PO					
Mean	258	678	5099	654	40
Med.	259	663	4865	649	40
S.D.	19.8	60.3	602.0	41.7	8.1
Min.	225	601	4505	611	27
Max.	282	793	6359	741	57
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-
FO Generation 5000 PPM Female PO					
Mean	254	679	4986	711	41
Med.	255	663	4841	709	40
S.D.	16.5	39.9	754.6	49.9	9.9
Min.	213	640	4220	648	22
Max.	273	774	6706	803	56
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	+	-
FO Generation 20000 PPM Female PO					
Mean	265	663	4357	650	46
Med.	266	665	4689	648	45
S.D.	15.5	44.6	997.0	89.4	7.5
Min.	234	587	2960	508	38
Max.	286	726	5409	794	60
N	10	10	10	10	10
TS 1%	-	-	-	-	-
TS 5%	-	-	-	-	-

Number of Viable Pups / Means
F0 Generation

		day 0	4 bef.	4 red.	4 aft.	7	14	21	28
0 PPM									
M	I	11.75	11.62	7.87	7.87	7.87	7.87		
s	I	1.83	1.84	0.35	0.35	0.35	0.35		
n	I	8	8	8	8	8	8		
1000 PPM									
M	I	11.20	11.20	7.90	7.90	7.90	7.80		
s	I	1.75	1.75	0.31	0.31	0.31	0.42		
n	I	10	10	10	10	10	10		
TS 1%	I	-	-	-	-	-	-		
TS 5%	I	-	-	-	-	-	-		
5000 PPM									
M	I	11.80	11.40	8.00	7.90	7.80	7.80		
s	I	1.61	1.57	0.47	0.56	0.63	0.63		
n	I	10	10	10	10	10	10		
TS 1%	I	-	-	-	-	-	-		
TS 5%	I	-	-	-	-	-	-		
20000 PPM									
M	I	8.87	9.85	7.28	7.28	7.28	7.28		
s	I	4.48	3.53	1.49	1.49	1.49	1.49		
n	I	8	7	7	7	7	7		
TS 1%	I	-	-	-	-	-	-		
TS 5%	I	-	-	-	-	-	-		

Litter Weight (g) / Means (g)
F0 Generation

		day					
		0	4 bef.	4 red.	7 aft.	14	21	28
0 PPM								
M	I	68.20	111.96	76.61	121.81	200.22	320.97	
s	I	10.12	18.25	9.07	13.36	19.29	32.36	
n	I	8	8	8	8	8	8	
1000 PPM								
M	I	66.42	109.35	78.12	121.48	200.35	314.41	
s	I	7.71	9.57	8.95	12.82	9.03	26.96	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	
5000 PPM								
M	I	65.28	100.27	71.98	110.63	179.42	289.40	
s	I	5.71	12.10	8.81	15.21	17.49	35.63	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	
20000 PPM								
M	I	51.30	94.60	72.58	110.77	181.92	288.52	
s	I	24.44	24.16	13.86	18.30	35.49	60.02	
n	I	8	7	7	7	7	7	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	

Body Weights of Pups / Means (g)
F0 Generation

		day					
		0	4 bef.	4 red. aft.	7	14	21	28
FEMALE PUPS								
0 PPM								
M	I	5.63	9.47	9.52	15.20	25.31	40.72	
s	I	0.42	0.91	0.92	1.22	3.19	5.35	
n	I	8	8	8	8	8	8	
1000 PPM								
M	I	5.79	9.70	9.71	15.05	25.01	39.82	
s	I	0.54	1.38	1.37	1.93	1.10	2.73	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	
5000 PPM								
M	I	5.39	8.72	8.81	13.53	22.60	36.50	
s	I	0.48	1.02	0.88	1.66	2.86	4.36	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	+	-	-	
20000 PPM								
M	I	5.77	9.95	10.04	15.30	25.29	39.57	
s	I	0.79	1.97	1.90	1.92	4.27	4.79	
n	I	7	7	7	7	7	7	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	

Body Weights of Pups / Means (g)
F0 Generation

		day 0	4 bef.	4 red.	4 aft.	7	14	21	28
MALE PUPS									
0 PPM									
M	I	6.00	9.85		9.86	15.61	25.48	40.77	
s	I	0.39	0.81		0.83	1.47	1.61	3.48	
n	I	8	8		8	8	8	8	
1000 PPM									
M	I	6.14	10.11		10.11	15.74	25.72	40.74	
s	I	0.58	1.39		1.42	1.65	1.73	2.78	
n	I	10	10		10	10	10	10	
TS 1%	I	-	-		-	-	-	-	
TS 5%	I	-	-		-	-	-	-	
5000 PPM									
M	I	5.73	8.93		9.10	14.27	23.37	37.55	
s	I	0.45	1.01		0.85	1.70	2.82	4.23	
n	I	10	10		10	10	10	10	
TS 1%	I	-	-		-	-	-	-	
TS 5%	I	-	+		-	-	-	-	
20000 PPM									
M	I	6.26	10.41		10.41	15.76	25.68	40.57	
s	I	0.93	1.89		1.86	1.89	3.86	4.39	
n	I	8	7		7	7	7	7	
TS 1%	I	-	-		-	-	-	-	
TS 5%	I	-	-		-	-	-	-	

Body Weights of Pups / Means (g)
F0 Generation

		day					
		0	4 bef.	4 red.	7 aft.	14	21	28
PUPS (TOTAL)								
0 PPM								
M	I	5.82	9.65	9.70	15.43	25.43	40.80	
s	I	0.43	0.86	0.84	1.24	2.23	4.11	
n	I	8	8	8	8	8	8	
1000 PPM								
M	I	5.99	9.94	9.92	15.40	25.38	40.31	
s	I	0.57	1.39	1.40	1.77	1.25	2.76	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	
5000 PPM								
M	I	5.58	8.86	8.98	13.98	23.11	37.16	
s	I	0.45	0.91	0.82	1.46	2.65	4.18	
n	I	10	10	10	10	10	10	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	
20000 PPM								
M	I	6.04	10.12	10.19	15.47	25.44	39.97	
s	I	0.84	1.90	1.85	1.88	4.15	4.64	
n	I	8	7	7	7	7	7	
TS 1%	I	-	-	-	-	-	-	
TS 5%	I	-	-	-	-	-	-	

Body Weights

(g)

012334/97.001

Anim. No.	Week 0	1	2	3	4	6	7	8	9	10	11	11	12	13
F0 Generation 0 PPM Male PO														
1	292	316	337	349	357									
2	289	310	328	346	360									
3	299	325	342	358	380									
4	291	318	329	337	355									
5	344	386	409	434	451									
6	340	377	394	404	413									
7	327	345	357	373	385									
8	284	311	336	355	368									
9	323	350	372	383	393									
10	326	341	359	377	396									
F0 Generation 1000 PPM Male PO														
21	291	314	321	336	344									
22	293	320	338	347	357									
23	292	314	327	343	358									
24	272	303	329	341	357									
25	275	292	315	324	341									
26	335	370	381	392	396									
27	310	345	373	387	406									
28	337	365	401	410	432									
29	291	326	343	360	379									
30	314	336	358	371	376									
F0 Generation 5000 PPM Male PO														
41	284	311	322	332	337									
42	289	319	340	359	361									
43	290	318	332	346	349									
44	265	294	311	327	339									
45	312	338	364	378	390									
46	345	380	418	437	454									
47	300	333	350	372	383									
48	324	364	384	411	422									
49	283	310	333	352	366									
50	298	329	353	372	383									
F0 Generation 20000 PPM Male PO														
61	302	322	340	354	364									
62	284	314	341	352	365									
63	266	304	328	348	363									
64	299	317	340	357	373									
65	282	294	319	326	336									
66	347	370	396	413	421									
67	308	327	351	363	373									
68	326	314	357	379	394									
69	305	339	368	376	385									
70	320	348	374	389	404									

Body Weights

(g)

Pregnancy | Lactation

115434/97.002

Anim. No.	0	1	2	3	4	Week 6	7	8	9	10	10	11	12	13
F0 Generation 0 ppm Female P0														
11	181	180	188	194	193	210	229	222	221					
12	174	176	173	186	186	184	211	241	302	213	233	236	215	240
13	173	186	198	204	204	200	222	223	234					
14	174	178	191	196	192	181	212	209	214	227	235	252	260	253
15	168	169	169	174	184	174	210	228	288	236	228	240	202	237
16	197	202	206	216	225	216	252	277	345	267	278	288	240	253
17	184	193	196	196	201	203	231	251	315	252	268	271	225	247
18	206	209	214	216	224	223	255	275	346	244	268	278	209	281
19	200	207	216	233	228	211	243	244	252	261	283	278	246	275
20	187	199	206	212	215					241	246	254	207	256
F0 Generation 1000 ppm Female P0														
31	168	182	191	201	196	192	219	224	226	217	230	245	256	260
32	178	181	181	193	200	185	215	240	291	230	235	258	226	222
33	164	177	177	181	185	183	208	227	289	202	234	236	212	224
34	187	196	207	213	214	216	235	259	317	239	257	263	222	258
35	172	183	187	196	195	189	215	236	305	214	235	242	201	228
36	192	199	210	217	213	201	235	233	254	238	251	265	234	236
37	188	198	202	213	216	209	239	264	341	233	241	250	225	257
38	198	206	209	219	218	209	236	256	325	224	246	256	215	273
39	207	212	217	221	232	228	264	285	350	263	285	287	236	277
40	199	207	216	227	231	231	268	298	371	278	286	292	255	282
F0 Generation 5000 ppm Female P0														
51	170	179	192	202	199	188	218	217	225	233	237	251	247	270
52	176	185	191	196	203	201	228	246	317	198	230	244	208	252
53	168	175	182	191	187	205	226	248	306	208	232	242	216	229
54	174	180	185	191	197	190	217	248	319	208	227	237	194	234
55	179	195	206	214	213	215	238	265	331	231	248	259	216	212
56	183	194	202	202	205	202	230	250	320	213	249	262	217	253
57	183	196	206	203	209	213	240	258	313	252	259	270	221	256
58	189	209	219	224	223	229	254	281	343	268	272	281	211	260
59	193	204	212	218	223	220	245	269	334	233	248	267	219	259
60	189	197	203	207	213	195	231	230	228	242	253	254	279	272
F0 Generation 20000 ppm Female P0														
71	181	187	201	207	212	202	244	233	255	240	256	265	221	277
72	180	188	201	206	206	204	232	252	288	242	250	257	251	258
73	186	188	203	206	205	198	233	236	250					
74	158	167	168	175	179	184	207	236	292	206	220	221	192	232
75	178	190	192	202	206	188	230	235	240	247	258	265		
76	200	211	226	233	241					266	277	294	237	244
77	185	197	198	203	211	210	223	230	239					
78	184	192	204	212	213	213	234	228	241	253	269	282	276	278
79	196	206	211	214	223	218	250	275	348	245	255	255	203	273
80	187	199	201	206	216	222	255	283	349	245	266	274	288	281

Food Consumption

(g/d)

012344/97.001

Anim. No.	Week 1	2	3	4
F0 Generation 0 PPM Male PO				
1	19.6	19.5	20.5	20.5
2	17.2	18.5	18.7	16.8
3	19.7	19.6	23.2	21.8
4	20.0	18.5	18.7	21.0
5	23.1	23.9	24.6	24.4
6	23.5	21.1	20.7	20.7
7	18.9	20.2	22.2	22.0
8	20.1	20.7	20.8	21.1
9	21.5	22.2	21.7	23.0
10	19.9	21.8	23.5	24.0
F0 Generation 1000 PPM Male PO				
21	20.4	16.8	27.0	22.7
22	19.5	20.3	22.4	21.0
23	22.0	18.7	21.5	20.7
24	18.5	17.4	19.2	20.4
25	19.4	19.0	20.4	21.2
26	26.1	21.1	24.9	23.6
27	20.4	22.8	22.1	23.5
28	22.1	23.5	25.2	23.4
29	19.9	17.7	22.3	21.8
30	20.9	20.0	22.0	21.0
F0 Generation 5000 PPM Male PO				
41	18.0	17.1	22.9	18.2
42	19.1	19.6	21.3	18.5
43	19.2	18.4	21.3	18.5
44	19.0	19.0	21.0	18.7
45	19.7	20.1	20.8	19.9
46	20.3	23.3	25.4	24.0
47	20.7	21.0	22.9	22.2
48	21.3	20.7	23.3	20.7
49	19.3	20.0	26.3	24.9
50	20.2	23.3	36.2	26.4
F0 Generation 20000 PPM Male PO				
61	18.5	19.7	22.0	20.8
62	18.7	19.7	21.0	21.6
63	19.2	19.7	27.2	25.8
64	20.6	21.3	22.7	22.8
65	18.1	21.8	21.2	21.0
66	21.1	21.5	24.9	23.3
67	18.1	21.9	23.0	22.1
68	20.0	22.3	25.6	25.6
69	19.4	22.8	23.6	21.8
70	20.1	23.2	24.4	23.5

Food Consumption

(g/d)

012344/97.002

Anim. No.	Week 1	2	3	4
F0 Generation 0 PPM Female P0				
11	12.2	11.7	14.1	11.9
12	17.9	10.7	23.6	14.5
13	14.4	18.5	42.9	31.0
14	11.9	12.6	15.4	15.1
15	12.4	11.2	15.8	14.1
16	14.8	13.5	22.3	17.4
17	13.3	12.6	14.4	13.3
18	14.4	13.0	17.3	14.5
19	16.8	14.4	27.2	14.5
20	16.4	15.0	16.8	17.6
F0 Generation 1000 PPM Female P0				
31	15.0	14.1	15.4	12.7
32	11.8	11.5	15.9	15.4
33	12.5	11.4	13.4	13.5
34	14.2	13.9	16.6	13.8
35	13.2	12.0	14.2	14.7
36	12.7	12.3	12.2	11.5
37	12.1	12.8	16.3	12.2
38	12.8	13.0	18.9	15.1
39	14.6	13.7	16.8	15.9
40	13.5	15.3	19.5	16.6
F0 Generation 5000 PPM Female P0				
51	12.6	12.9	13.5	12.2
52	15.6	16.3	25.8	17.4
53	12.0	15.3	21.4	14.5
54	11.7	11.4	12.3	12.2
55	14.4	14.7	16.5	15.5
56	26.8	14.8	20.4	14.9
57	13.3	12.8	14.4	14.5
58	15.6	14.7	19.8	15.6
59	13.9	14.3	16.2	15.1
60	13.9	13.9	18.1	15.0
F0 Generation 20000 PPM Female P0				
71	18.1	15.2	16.5	17.3
72	12.6	15.3	15.9	14.9
73	13.8	14.3	13.8	14.1
74	12.6	12.4	18.1	15.6
75	12.1	12.6	16.1	14.2
76	18.8	16.3	26.1	21.0
77	13.2	13.5	14.8	14.6
78	19.1	15.1	19.0	14.6
79	14.2	14.4	16.9	16.2
80	14.5	12.4	17.0	18.1

Duration of Pregnancy of F0-Females (Days)

Dose	0 ppm		1000 ppm		5000 ppm		20000 ppm
Animal No.		Animal No.		Animal No.		Animal No.	
11	§	31	#	51	#	71	#
12	22	32	23	52	23	72	22
13	§ *	33	22	53	23	73	§ *
14	#	34	23	54	23	74	22
15	21	35	22	55	22	75	#
16	22	36	#	56	23	76	$
17	22	37	22	57	22	77	§ *
18	22	38	22	58	22	78	#
19	#	39	22	59	22	79	22
20	$	40	22	60	#	80	23
Mean	21,8		22,3		22,5		22,3
Females with living pups	8		10		10		8

§ Insemination established, but animal was not pregnant
$ Insemination not established, but animal was pregnant
# Second mating procedure. Duration of pregnancy not to be established
§ * Insemination established, but animal was not pregnant including second mating procedure

Absolute Organ Weights

013564/97.001

Anim. No.	Body W. G	Brain mg	Liver mg	Kidneys mg	Testes mg
F0 Generation 0 PPM Male PO					
1	429	1919	14657	2214	3524
2	421	1886	15069	2398	3297
3	443	1905	12911	2440	3570
4	433	2056	13356	2509	3570
5	507	1995	16475	2802	3701
6	476	1938	16193	2701	3373
7	419	1897	14441	2677	3436
8	446	1964	14277	2643	2870
9	450	1974	16283	2874	3566
10	456	2055	17688	2678	3359
F0 Generation 1000 PPM Male PO					
21	412	1980	14387	2431	3423
22	410	1995	16006	2660	3174
23	421	1935	13659	2458	3376
24	404	1948	14120	2273	3139
25	416	1977	13142	2354	2949
26	487	1988	17337	2301	3437
27	465	1922	15088	2502	3458
28	527	2154	18009	2594	3683
29	445	2097	14894	2428	3386
30	438	1946	15545	2636	3680
F0 Generation 5000 PPM Male PO					
41	416	1869	13604	2179	3963
42	416	1905	15530	2453	3160
43	399	2017	12919	2259	3421
44	422	1932	15060	2349	2953
45	453	1975	16068	2625	4393
46	503	1971	17096	2692	3471
47	452	1887	16020	2261	3536
48	497	2037	16813	2592	3069
49	443	1912	16055	2204	2987
50	462	1862	14690	2081	3350
F0 Generation 20000 PPM Male PO					
61	421	1953	12625	2171	3761
62	428	1909	14409	2497	3657
63	458	1855	14057	2589	3147
64	437	2036	14892	2558	3103
65	396	1766	13072	2199	3344
66	475	2016	15921	2811	3488
67	447	1935	14092	2680	3551
68	471	2055	14932	2603	3629
69	432	1825	12362	2256	3684
70	474	2033	16056	2655	3931

Absolute Organ Weights

013564/97.002

Anim. No.	Body W. G	Brain mg	Liver mg	Kidneys mg	Ovaries mg
F0 Generation 0 PPM Female P0					
11	237	1821	8066	1568	147
12	240	1739	9809	1602	96
13	245	1828	7806	1335	150
14	254	1704	10674	1633	152
15	237	1731	10579	1537	92
16	254	1786	9959	1715	85
17	247	1742	12217	1557	89
18	282	1789	16691	1584	83
19	292	1912	14510	1853	98
20	256	1721	12856	1569	123
F0 Generation 1000 PPM Female P0					
31	261	1692	12134	1744	149
32	246	1808	11087	1504	100
33	225	1783	12482	1551	81
34	257	1817	11940	1687	116
35	229	1657	14562	1698	81
36	273	1679	12511	1694	108
37	258	1737	12755	1592	88
38	274	1784	15234	1857	74
39	278	1759	15012	1710	114
40	282	1696	13480	1814	126
F0 Generation 5000 PPM Female P0					
51	270	1730	12508	1766	129
52	253	1671	12389	1787	80
53	252	1698	12957	1630	129
54	249	1798	16724	1782	56
55	213	1645	8968	1706	118
56	254	1678	10758	1761	103
57	256	1742	14714	1724	100
58	261	1728	13055	1908	95
59	260	1726	11660	2018	93
60	273	1769	13013	1933	127
F0 Generation 20000 PPM Female P0					
71	270	1803	14243	2142	123
72	258	1874	10925	1906	110
73	262	1842	7755	1499	133
74	234	1628	12168	1661	106
75	260	1810	7907	1464	150
76	286	1773	15487	1808	115
77	250	1654	7527	1272	150
78	279	1709	12755	1851	111
79	273	1604	13804	1664	103
80	278	1837	13327	1974	125

Relative Organ Weights

Relative to Body Weights

015754/97.001

Anim. No.	Body W. G	Brain mg/100g	Liver mg/100g	Kidneys mg/100g	Testes mg/100g
F0 Generation 0 PPM Male P0					
1	429	447	3417	516	821
2	421	448	3578	569	783
3	443	430	2914	551	806
4	433	475	3085	580	825
5	507	394	3251	553	730
6	476	407	3402	567	709
7	419	453	3449	639	821
8	446	440	3202	593	644
9	450	438	3615	638	792
10	456	451	3883	588	737
F0 Generation 1000 PPM Male P0					
21	412	481	3494	590	831
22	410	487	3907	649	775
23	421	460	3247	584	802
24	404	483	3499	563	778
25	416	476	3162	566	710
26	487	408	3558	472	705
27	465	413	3242	538	743
28	527	409	3419	493	699
29	445	471	3345	545	761
30	438	444	3547	602	840
F0 Generation 5000 PPM Male P0					
41	416	450	3274	524	954
42	416	458	3738	590	761
43	399	505	3236	566	857
44	422	458	3571	557	700
45	453	436	3545	579	969
46	503	392	3401	536	690
47	452	418	3547	501	783
48	497	410	3383	522	618
49	443	432	3624	498	674
50	462	403	3177	450	724
F0 Generation 20000 PPM Male P0					
61	421	464	3002	516	894
62	428	446	3370	584	855
63	458	405	3067	565	687
64	437	466	3412	586	711
65	396	447	3305	556	846
66	475	425	3352	592	734
67	447	433	3152	599	794
68	471	437	3173	553	771
69	432	423	2865	523	854
70	474	429	3390	561	830

Relative Organ Weights

Relative to Body Weights

015754/97.002

Anim. No.	Body W. G	Brain mg/100g	Liver mg/100g	Kidneys mg/100g	Ovaries mg/100g
F0 Generation 0 PPM Female PO					
11	237	770	3411	663	62
12	240	724	4084	667	40
13	245	746	3187	545	61
14	254	671	4204	643	60
15	237	729	4456	647	39
16	254	704	3927	676	34
17	247	704	4938	629	36
18	282	635	5921	562	29
19	292	656	4976	635	34
20	256	671	5016	612	48
F0 Generation 1000 PPM Female PO					
31	261	649	4656	669	57
32	246	735	4505	611	41
33	225	793	5550	690	36
34	257	706	4640	656	45
35	229	724	6359	741	35
36	273	614	4576	620	40
37	258	674	4950	618	34
38	274	652	5566	678	27
39	278	634	5410	616	41
40	282	601	4780	643	45
F0 Generation 5000 PPM Female PO					
51	270	640	4626	653	48
52	253	662	4907	708	32
53	252	675	5148	648	51
54	249	721	6706	715	22
55	213	774	4220	803	56
56	254	661	4240	694	41
57	256	680	5743	673	39
58	261	662	5002	731	36
59	260	665	4492	777	36
60	273	649	4775	709	47
F0 Generation 20000 PPM Female PO					
71	270	668	5279	794	46
72	258	726	4231	738	43
73	262	703	2960	572	51
74	234	697	5211	711	45
75	260	695	3036	562	58
76	286	619	5409	632	40
77	250	661	3008	508	60
78	279	614	4580	665	40
79	273	587	5053	609	38
80	278	662	4799	711	45

Number of Viable Pups
F0 Generation 0 PPM

Dam No.	I I I	day 0	4 bef. red.	4 aft.	7	14	21	28
12		12	11	7	7	7	7	
14		12	12	8	8	8	8	
15		10	10	8	8	8	8	
16		12	12	8	8	8	8	
17		9	9	8	8	8	8	
18		13	13	8	8	8	8	
19		15	15	8	8	8	8	
20		11	11	8	8	8	8	

Number of Viable Pups
F0 Generation 1000 PPM

Dam No.	I I I	0	4 bef.	4 red.	4 aft.	7	14	21	28
					day				
31		13	13		8	8	8	8	
32		7	7		7	7	7	7	
33		11	11		8	8	8	8	
34		10	10		8	8	8	8	
35		12	12		8	8	8	7	
36		12	12		8	8	8	8	
37		13	13		8	8	8	8	
38		12	12		8	8	8	8	
39		11	11		8	8	8	8	
40		11	11		8	8	8	8	